PISMO BEACH FECAL CONTAMINATION SOURCE IDENTIFICATION STUDY

FINAL REPORT

Prepared for:

THE CITY OF PISMO BEACH

California Clean Beaches Initiative:

Contract # 07-578-550-2

Project # 508

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I. Executive Summary

This project began when the San Luis Obispo County Public Health Department noticed increases in the number of times warnings for high bacteria levels had to be posted for Pismo Beach. The City of Pismo Beach (CPB) applied for funding from the California State Water Resources Control Board (CSWRCB) under the Proposition 50 Clean Beaches Initiative. The Environmental Biotechnology Institute (EBI) at California Polytechnic State University, San Luis Obispo (Cal Poly) was subcontracted to run the project. Matching funds from both Cal Poly and CPB were added to the funds made available by Proposition 50.

The primary goal of the project was to identify the biological sources of fecal contamination as well as the physical and environmental factors that influence the levels of bacteria in the ocean waters at Pismo Beach, California. Water samples were collected from 3 sites extending up Pismo Creek, 10 sites along the beach bracketing Pismo Beach pier, 5 sites in the ocean off Pismo Beach and one site over the joint Pismo/Arroyo Grande/Oceano wastewater outfall to the south of Pismo Beach (Table 4.2-1, Figures 4.2-1 and 4.5-1.). Samples were tested for the presence and abundance of fecal indicator bacteria (FIB) as well as a variety of tests designed to detect bacteria that could serve as indicators of the biological source of fecal contamination. Physical, chemical and environmental data, including wind speed and direction, tide height, cloud cover, water temperature, salinity, turbidity, wave height, ocean current and more, were also collected during sampling. Four sampling frequencies were utilized to maximize data coverage in the highly dynamic environment of an ocean beach: hourly, daily, weekly and rain event sampling. In addition, a 60-day volunteer monitoring program was initiated during the summer of 2008 to count visible fecal material on the beach and monitor visitor activity.

The data collected in this study clearly shows the main source of fecal contamination on the beach is bird droppings near the pier. Nearly 40% of the *E. coli* strains collected in this study matched bird fecal sources (Table 6.4.6-1 and 6.4.6-2), and *E coli* strains with a pigeon-specific fingerprint were collected twelve times from within 150 meters of the pier (section 6.4.6). FIB counts along the beach were clearly highest near the pier and dropped off with distance from the pier. Volunteer observations found the highest count of bird droppings within 100 meters of the pier and one observer at the pier estimated the size of the Pismo Beach pigeon flock at well over 400 birds with more than 200 pigeon nests in the structural members of the pier itself. Correlations to oceanographic conditions also corroborate this conclusion. Both wave direction and current direction worked to push high concentrations of FIB away from the pier as the main source of fecal contamination. In addition, measuring the time since a tide last washed the part of the beach being sampled was an excellent predictor of FIB count, indicating that deposition of fecal matter on the beach itself was a predominate contamination mode.

These key pieces of information, in unison, present a convincing argument for the pigeon flock at the Pismo Beach pier as the main source of fecal contamination in the surrounding ocean water. We suggest that the City of Pismo Beach find a way to reduce or remove the pigeon population that has taken up residence at the pier.

The project also had some secondary goals. Several different methods for fecal source tracking were used in the study and we provided a comparison and recommendations for future use of source tracking methodology at California beaches. Terminal Restriction Fragment Length

Polymorphism analysis and detection of horse-specific *Bacteroides* and human-Enterovirus were all shown to be insufficiently sensitive for determining sources of fecal contamination in the ocean. However, both the use of massive *E. coli* library matching and other host-specific *Bacteroides* tests provided good information.

The *E. coli* library matching study provided the only direct evidence of bird fecal influences on FIB counts. Almost 40% of the *E. coli* strains collected matched a bird fecal source, and 20% of the *E. coli* collected matched a dog source. Many different fecal sources for *E. coli* in the ocean waters were also identified with this method, although our quality control experiments suggest that not all the sources identified were correct. Tests for human-, dog-, and cow-specific *Bacteroides* markers were used to good effect. As expected, evidence of cow fecal contamination was common in the creek samples taken during rain events, was only rarely seen in beach samples, and almost never observed in samples taken near the pier. While many samples were positive for human- and dog-specific *Bacteroides*, indicating that both human and dog feces are making it into the ocean at Pismo Beach, we found no evidence for dog or human influence on FIB counts. In addition, these assays were sensitive enough to detect less than a tenth of a gram of fecal matter in a liter of ocean water, far less than what is required to detect FIB from the same source. Samples positive for dog feces were more common on the weekends while samples positive for human feces were more common in the middle of the week. In addition, most of the beach samples in a five-day window on each side of the July 4th holiday in 2008 tested positive for human-specific *Bacteroides* – even extending to samples taken from the ocean beyond the surfzone.

To mitigate the issues associated with dog and human sources of fecal contamination we suggested increased restroom access for swimmers, especially during high beach visitor times and an increased presence on the beach to enforce dog dropping pickup laws more strictly or higher fines for failure to comply.

Another secondary goal included reporting on the detection and enumeration of a set of pathogens known to cause problems in recreational waters. Seven bacterial pathogens (*Aeromonas* spp., *Campylobacter* spp., *Pseudomonas* spp., *Pseudomonas aeruginosa*, *Salmonella* spp., *Shigella* spp., *Vibrio parahaemolyticus*, and *Vibrio vulnificus*) and two protozoan parasites (*Giardia* and *Cryptosporidium*) were monitored in water taken next to the Pismo Beach pier and from the lagoon at the terminus of Pismo Creek. All pathogens we tested for were found in the Pismo Creek lagoon and next to the pier on the beach. In many cases, the amount of pathogens in the samples would require ingestion of large volumes of seawater to risk infection, but some pathogens clearly presented a risk at the levels we detected. Pathogen levels at PB4 (Table 4.2-1.) were rarely high and significantly lower than in the lagoon so the risk of disease from swimming next to the pier could be orders of magnitude lower. Pigeon feces were shown to harbor some of the pathogens we tested for, however, not all pathogens we tested for were correlated with high FIB counts. In fact, the two most common pathogens found in pigeon feces, *Aeromonas* spp. and *Pseudomonas* spp., were not correlated to FIB counts at all. Perhaps these bacteria die off in seawater at a different rate than do FIB. Interestingly, levels of *Campylobacter* spp., a pathogen known to be carried by birds, correlated well with FIB counts. However, very low levels of *Campylobacter* spp. were found at PB4 (Table 4.2-1.) and pigeons

do not appear to be common carriers. Still, it may be prudent to post the dangers of swimming in the Pismo Creek lagoon to ensure the public is informed of the health risks.

The last secondary goal for the project involved the development of a non-expert, hand-held, rapid sample preparation and testing method for detecting human fecal contamination in beach water samples. The subcontractor in charge of this effort, Advanced Liquid Logic, made good progress toward building a kit for the rapid detection of human *Bacteroides* in seawater, but about another year of work would be required before such a kit could be brought to market.

II. Abbreviations, Units and Terms

AB411 – California Assembly Bill number 411: An act to amend Sections 115880, 115885, and 115915 of the Health and Safety Code, relating to public beaches.

ADA-V – Ampicillin Dextrin Agar supplemented with Vancomycin, a microbial growth medium

AHB – Abeyta-Hunt Bark, a microbial growth medium

ALL – Advanced Liquid Logic in Morrisville, North Carolina (a subcontractor to EBI)

APHA – American Public Health Association

APW - Alkaline Peptone Water, a microbial growth medium

AUV – Autonomous Underwater Vehicle

AWAC – Acoustic Wave And Current profiler

BAM – Bacteriological Analytical Manual

BBB – Bad Bugs Book, an FDA web publication

BS – Bismuth Sulfite, a microbial growth medium

Cal Poly – California Polytechnic State University, San Luis Obispo

CCMS – Center for Coastal Marine Sciences at Cal Poly State University

cDNA – DNA copied from RNA, "copy DNA"

cDOM – Colored Dissolved Organic Material

CIPC – competitive internal positive control

CFU – Colony Forming Units (a way to count bacterial numbers)

CPB – City of Pismo Beach

CSWRCB – California State Water Resources Control Board

C_T – cycle threshold, in reference to a detection event used in qPCR

DQO – Data Quality Objectives

E. coli – *Escherichia coli*, specifically with reference to counts made by IDEXX method

EBI – Environmental Biotechnology Institute at Cal Poly State University

Ent – Enterococcus, specifically with reference to counts made by IDEXX method

EPA – Environmental Protection Agency

FC – Fecal coliform, specifically with reference to counts made by IDEXX method

FDA – Food and Drug Administration

FIB – Fecal Indicator Bacteria

FST – Fecal Source Tracking

g – grams

IEH – Institute for Environmental Health in Seattle, Washington (a subcontractor to EBI)

km – kilometers

L – liters

m – meters

mCPC – Modified Cellobiose-Polymyxin B-Colistin, a microbial growth medium

MDS – Multi-Dimensional Scaling

MF – Membrane Filtration

mFC – modified Fecal Coliform, , a microbial growth medium

mL – milliliters

M-PA-C – Modified *Pseudomonas aeruginsa* agar C, a microbial growth medium

MPN – Most Probable Number

MSL – Mean Sea Level

µm – micrometer

NA – Nutrient Agar, a microbial growth medium

PCR – polymerase chain reaction, a method for amplifying DNA

PBS – Phosphate Buffered Saline

QA – Quality Assurance

QAPP – Quality Assurance Project Plan

QC – Quality Control

qPCR – quantitative polymerase chain reaction, a method for quantifying amounts of DNA

REMUS – Remote Environmental Measuring UnitS

RV – Rappaport-Vassiliadis, a microbial growth medium

SC – Selenite Cystine, a microbial growth medium

SLO-CPHD – San Luis Obispo County Public Health Department

SOP – Standard Operating Procedure

SWRCB – State Water Resources Control Board

TC – Total Coliform, specifically with reference to counts made by IDEXX method

TCBS – Thiosulfate-Citrate-Bile Salts-Sucrose, a microbial growth medium

TSAMS – Trypticase Soy Agar-Magnesium sulfate-NaCl, a microbial growth medium

VPSA – Vibrio Parahaemolyticus Sucrose Agar, a microbial growth medium

XLD - Xylose Lysine Desoxycholate, a microbial growth medium

1. Introduction and Overview

This report describes the experimental procedures, data collected and interpreted and conclusions drawn from those data during a study of water quality at and around the Pismo Beach pier pursuant to grant agreement #08-052 between the City of Pismo Beach and The California Polytechnic State University, San Luis Obispo (Cal Poly). The project was funded by the Proposition 50 Clean Beaches Grant Program and a grant agreement between the State Water Resources Control Board (SWRCB) and the City of Pismo Beach agreement # 07-578-550-2. This study was conducted by faculty and staff of the Environmental Biotechnology Institute (EBI) and the Center for Coastal Marine Sciences (CCMS), both at Cal Poly. It also included subcontracted work performed by Applied Liquid Logic, Morrisville, North Carolina (ALL) and the Institute for Environmental Health, Seattle, Washington(IEH).

1.1. The Problem at Pismo Beach

Pismo Beach is an ocean beach extending from about ¾ of a mile north to about 6 miles south of the Pismo Beach pier, and is contiguous with a long stretch of beach leading south through Grover and Oceano Beaches down through the Pismo Dunes Natural Preserve (Figure 1.1-1). About ½ mile south of the pier, Pismo Creek forms a small lagoon before emptying into the ocean during the rainy season. Very little if any creek flow over the beach is visible for most of the dry season. Since the San Luis Obispo County Public Health Department (SLO-CPHD) began testing water quality in 2001, under California Assembly Bill 411 (AB411), Pismo Beach in the vicinity of the Pismo Beach pier has experienced increasing numbers of beach bacterial advisories during the summer months. As a result, Pismo Beach is on the Clean Beaches Task Force list of Priority Beaches. The City of Pismo Beach (CPB) is typical of many CA beach towns in that the majority of its business comes from the beach. Increased frequency and length of advisory postings could result in fewer visitors to the beach and decreased tourist-related income for the city as well as increased worries about the health of the city's residents and visitors. Consequently, the CPB is interested in determining the source of these high Fecal Indicator Bacteria (FIB) levels and, with an intent to eliminating these summer beach advisory postings, funding was requested for a microbial source tracking study.

Figure 1.1-1. Map indicating the position of the Pismo Beach pier in relation to the coast of California.

2. Project Summary

2.1. Objectives

The primary goal of this project was to identify the biological sources of fecal contamination as well as the physical and environmental factors that influence the levels of bacteria in the ocean waters at Pismo Beach, California. Water samples from selected locations were tested for the presence and abundance of microbes associated with fecal pollution and the source of the fecal contamination was determined. Physical and environmental data was also collected during sampling to examine the effects of these factors on fecal pollution. The focus of the study was the beach around the Pismo Beach pier although samples were taken further south along the beach where Pismo Creek enters the ocean and in the creek itself. These data were used to recommend a remediation plan for Pismo Beach, identify reasonable water quality goals and provide suggested methods for reaching those goals.

The project also had secondary goals. First, this project utilized several methods for fecal source tracking and then compared and contrasted these methods, making recommendations for future

use of source tracking methodology at California beaches. Perspectives on efficiency, cost and usefulness of data for remediation outcomes are detailed herein. Part of this goal included validation of these methods with site-specific samples. Second, this project included the detection and enumeration of a set of pathogens known to cause public health problems in recreational waters. Correlations between pathogen incidence and FIB counts as well as the sources of fecal bacteria were noted. The repercussions of this information on the use of traditional FIB counts for water quality are discussed in section 8.2 of this report. Last, this project included an effort to develop a rapid sample preparation and testing method for detecting human fecal contamination in beach water samples. The goal was to create a non-expert use assay that can be completed in less than one hour and uses equipment easily affordable to small beach communities in California.

2.2. History and Baseline Study Results

Historical data of FIB counts collected by the (SLO-CPHD) at three sites on Pismo Beach (Figure 2.2-1.), rainfall (CPB Sewage Treatment Facility) and tide levels (NOAA online tide database, Port San Luis Station) dating from January 2004 to May 2007 were analyzed to obtain a preliminary list of probable sources for the FIB levels that caused beach advisories and to develop hypotheses to guide the design of this FIB source study work plan. In addition, a preliminary baseline study was launched in the summer of 2007 to help design a sampling plan for the study.

Figure 2.2-1. Pismo Beach sampling stations monitored weekly by SLO-CPHD.

2.2.1. Previous Water Quality Data from San Luis Obispo County

FIB data from Pismo Beach was organized by the number of times an advisory was posted (an AB411 limit exceedence) and then by how many total days advisory postings were in effect. As is the case for most California beaches, there were more advisories posted and more posting days total during the rainy season (October through April) than during the dry season (Figure 2.2.1-1). In addition, there was a trend toward more postings and a higher number of posted days at the PB4 sampling site, 40 feet south of the Pismo Beach pier. Of particular relevance to the tourist industry at Pismo Beach, dry season advisories were predominantly posted due to exceedences at the PB4 sampling site. Lastly, increased rainfall in 2005-2006 was followed by longer postings and postings earlier in the 2006 dry season.

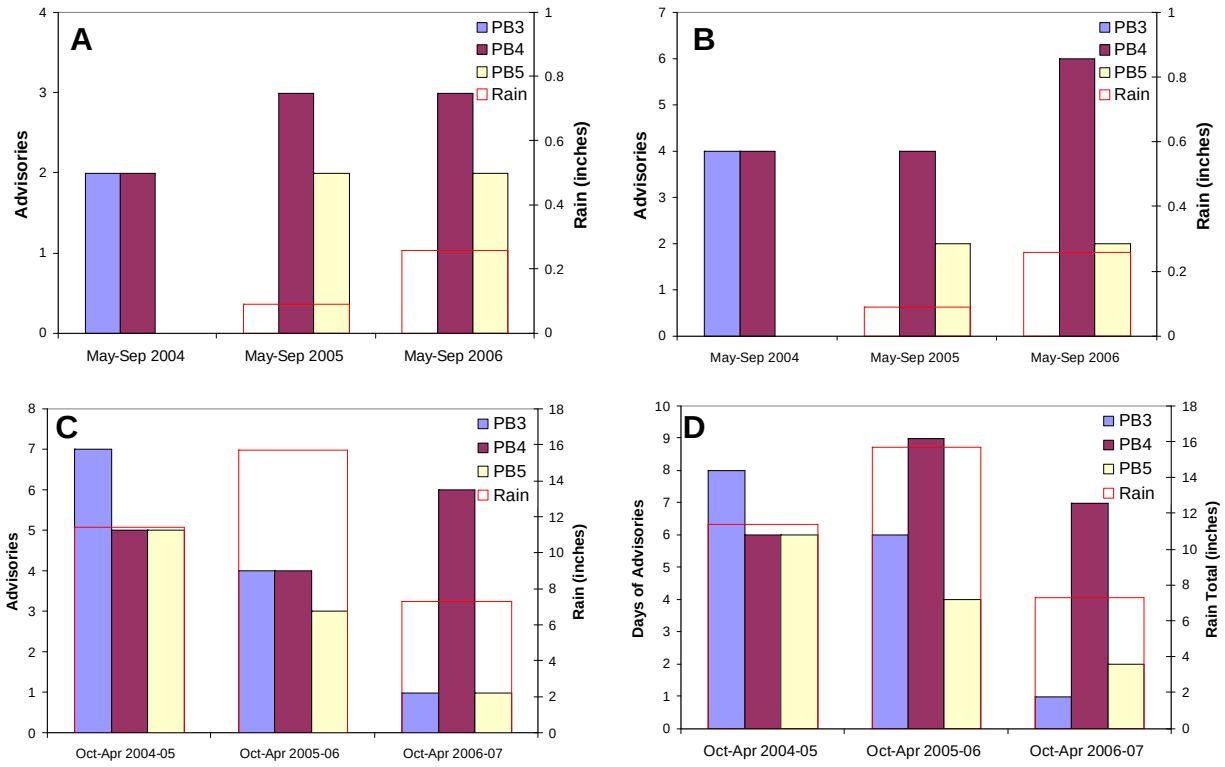


Figure 2.2.1-1. Frequency of beach advisories from 2004 to 2007 for the three sampling areas at Pismo Beach monitored by SLO-CPHD: A) number of advisories posted during dry seasons; B) total advisory posting days during dry seasons; C) number of advisories posted during rainy seasons; D) total advisory posting days during rainy seasons.

FIB counts were then separated by type and summed over each month of sampling to look for differences in the type of bacteria causing an advisory posting (Figure 2.2.1-2). During the rainy season, *Enterococcus* (Ent) were the predominant cause of advisory postings and levels were fairly consistent across all three sites. A cursory study of rainfall events in Pismo Beach showed that most exceedences occurring during the rainy season were correlated with rainfall of at least 0.5 inches/day (data not shown). Conversely, fecal coliforms (FC) were the predominant cause

of advisory postings during the dry season, particularly in August and particularly at the PB4 site.

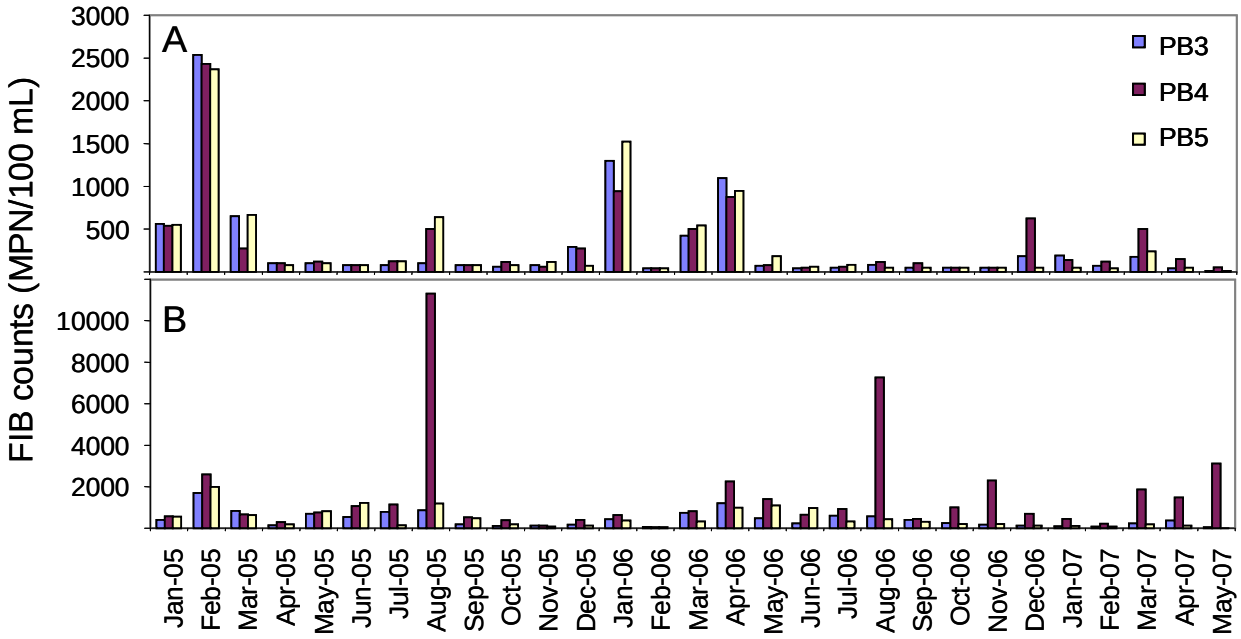


Figure 2.2.1-2. FIB counts by month for 2004-2007 data: A) Ent counts and B) FC counts.

Finally, FC counts were graphed with tide level data from the Port San Luis pier over the 2005 and 2006 dry seasons (no tide data was available for August 2004). This analysis was only performed for FC counts because dry season exceedences were due to FC levels. In most cases, FC counts that exceeded health limits occurred within a few days of the peak in the 14-day spring tide cycle (Figure 2.2.1-3). This trend played out again in the summer of 2007 with an advisory posting July 3rd 2007, the first sampling after the spring tide on June 30th and another July 16th, the first sampling after the spring tide on July 13th (Rich Lichtenfels, SLO-CPDH, personal communication).

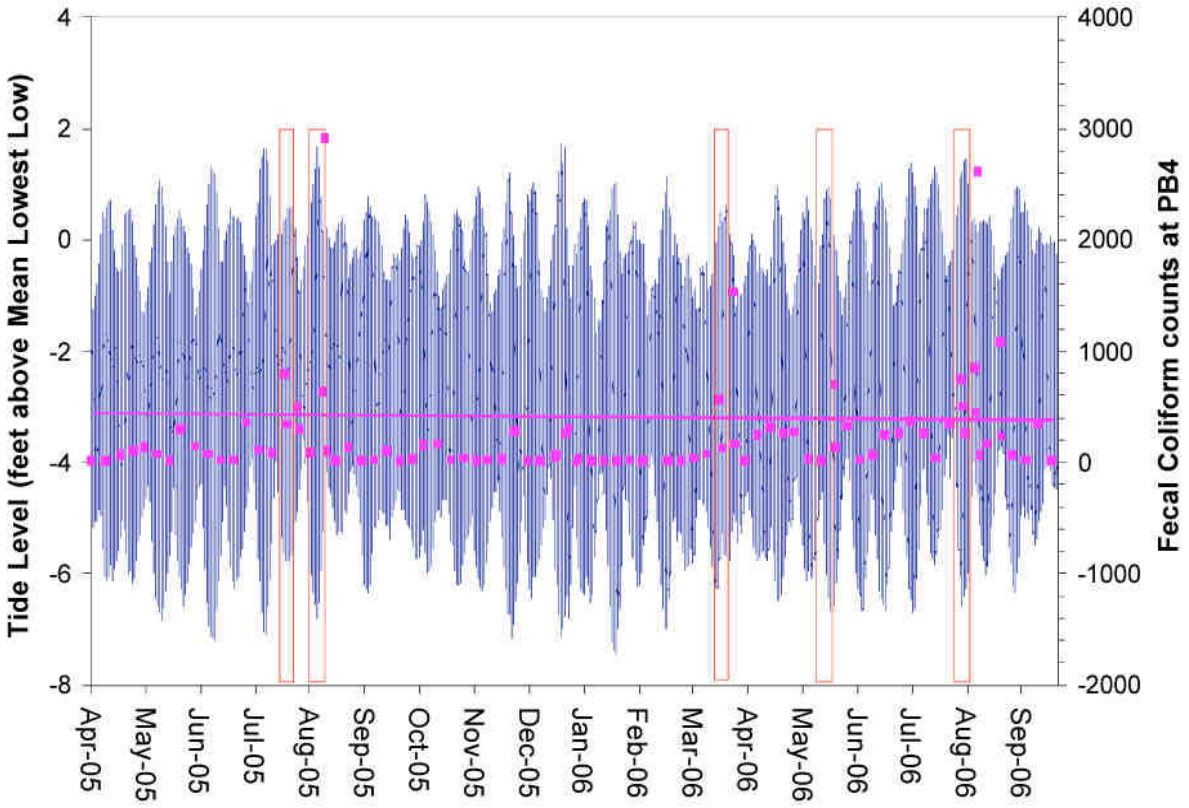


Figure 2.2.1-3. FC counts and tide levels. Pink squares indicate FC counts (MPN/100 mL) and blue lines indicate tide levels. The pink horizontal line is the recreational water advisory limit. Note that the majority of counts high enough to result in beach advisories are close to the peak of the 14-day spring tide cycle (red boxes).

2.2.2. Baseline Study During Summer 2007

Two sampling plans were implemented for a baseline study conducted over the summer 2007 before a Quality Assurance Project Plan (QAPP) had been completed for the entire study. The sampling sites included the PB3, PB4 and PB5 sites routinely sampled by SLO-CPHD as well as sites in between (PB 4.5 and PB3.5), sites further south (PB2 and PB1) and one site in the Pismo Lagoon (L1). GPS data for all sites used in the study are provided in section 4.2 below (Table 4.2-1). The first sampling plan covered 10 sites for daily sampling from August 1st through August 30th 2007. A total of 36 out of the 300 samples collected (12%) exceeded the AB411 standards for beach water quality with either Total Coliform (TC), Enterococcus(Ent), *Escherichia coli* (*E. coli*)or a combination of these FIB counts (Table 2.2.2-1). Ninety percent of samples from the lagoon site (L1) exceeded AB411 limits. Excluding L1, only 6.6% of the samples taken exceeded AB411 limits. Exceedences were highest at the PB4 site (43%) 12 meters south of the pier and lowest at PB2 (0%), 600 meters south of the pier. This data was used to design an improved daily sampling plan for the summer of 2008 that dropped L1 and

included additional sites closer to the pier to better pinpoint physical sources of FIB on the beach.

Table 2.2.2-1. Frequency of AB411 exceedences in the daily sampling over 30 days in the summer of 2007, broken out by sampling site. Totals may not appear additive if more than one FIB resulted in an exceedence for the same day.

Site Name	TC	Ent	*E. coli*	Total
PB5	0	3	2	3
PB4.5	0	1	1	1
PB4	1	1	13	13
PB3.5	0	5	2	5
PB3	0	1	1	2
PB2	0	0	0	0
PB1	0	1	0	1
O4	0	0	0	0
O4.1	0	0	2	2
L1	15	16	11	27
Total	16	28	32	36
Total without L1	1	9	18	19

The second sampling plan in the baseline study included five sites centered on the pier (Table 2.2.2-2) with hourly samples taken from 4 am August 10th 2007 until 3 am August 11th 2007. AB411 exceedences appeared to correlate with incoming and peak tides. This data was used to plan for two 48-hour, hourly sampling plans for the summer of 2008 to better characterize the effect of tides on FIB counts.

Table 2.2.2-2. AB411 exceedences (**X**) in the 24-hour sampling over summer of 2007, broken out by sampling site and sampling time. Tide height is noted when counts exceeded AB411 limits. Adjacent sampling times with no AB411 exceedences at any site were combined in rows with tidal trend indicated to save space.

	PB3		PB3.5		PB4		PB4.5		PB5		
Date & Time	**Ent**	***E. coli***	**Ent**	***E. coli***	**Ent**	***E. coli***	**Ent**	***E. coli***	**Ent**	***E. coli***	**Tide Height (m) or Trend**
8/10/2007 4am -5am	.	.	.	.	.	.	.	.	.	.	increasing
8/10/2007 6am	.	**X**	.	.	.	.	.	.	.	.	0.243
8/10/2007 8am	.	.	**X**	.	**X**	.	.	.	**X**	**X**	0.907
8/10/2007 9am	.	.	.	.	.	.	.	.	**X**	.	1.131
8/10/2007 10am - 2pm	.	.	.	.	.	.	.	.	.	.	decreasing
8/10/2007 3pm - 8pm	.	.	.	.	.	.	.	.	.	.	increasing
8/10/2007 9pm	**X**	.	**X**	.	**X**	.	.	.	.	.	1.951
8/10/2007 10pm	**X**	.	.	.	.	.	**X**	.	.	.	1.811
8/10/2007 11pm	.	.	.	.	.	.	.	.	**X**	.	1.499
8/11/2007 12am	**X**	**X**	.	.	.	.	.	.	.	.	1.065
8/11/2007 1am	.	.	.	.	.	.	.	.	.	.	0.585
8/11/2007 2am	.	.	.	.	.	.	**X**	.	**X**	.	0.151
8/11/2007 3am	.	.	.	.	.	.	.	.	.	.	-0.155

2.3. Hypotheses and Potential Fecal Sources

These data combined with anecdotal information on local ocean currents and flow patterns for Pismo Creek resulted in the following hypotheses for physical/spatial/temporal sources of FIB counts causing bacterial advisories. During the rainy season, the largest loads of FIB may originate from Pismo Creek and/or storm water coming onto the beach during rain events. Conversely, during the dry season, the largest loads of FIB may originate from the beach itself as they are washed into the surf zone at the highest high tides; mostly in the area of the pier. These

hypotheses for physical/spatial/temporal sources of FIB along with anecdotal information on tourist behavior, bird populations and land use result in corollary hypotheses as to the biological sources for FIB at Pismo Beach. During the rainy season most FIB may come from human and domestic animal (dog, cow, horse) sources of feces washed into the surf zone during rain events. During the dry season most FIB may come from dog, human, horse, or bird feces directly on the beach that are washed into the surf zone at the highest concentration during the highest high tides. This study addresses these hypotheses directly. Other possible physical/biological sources of FIB include the joint Pismo/Grover/Oceano sewage outfall, marine mammals, wild animals and/or human encampments in the Pismo Creek watershed. The study addresses some of these sources, although they do not seem likely to be major contributors to FIB causing bacterial advisory postings given the preliminary data analysis above.

2.4. Funding Summary

California State funds from the Proposition 50 Clean Beaches Initiative provided the majority of funding for this project. Cal Poly provided $36,048 for the installation of an Acoustic Wave And Current (AWAC) profiler off the end of the Pismo Beach pier for use during the study (section 4.8). This device was also used by the Southern California Coastal Ocean Observation System (SCCOOS, www.sccoos.org) program in a section administered by CCMS.

The CPB also provided funding in the form of a 15% match applied to the portion of the Project that was considered capital costs: $31,233 toward personnel services, $3,845 in operating costs and $63,717 toward Professional and Consultant Services as defined under Section 32025 of the Public Resources Code.

Early funding to implement the baseline study was supplied by the EBI at Cal Poly and then reimbursed by the State. Funding for Proposition 50 projects was frozen in December 2008 and the EBI supplied funds to continue the sampling plan. These funds were also reimbursed by the SWRCB when funding was reinstated for this project in December 2009. The total amount invoiced to the state is $533,672. The total funding, not including the $36,048 contributed by Cal Poly, is $559,208 (Table 2.4-1).

Funding for this project has been provided in full or in part through an agreement with the State Water Resources Control Board. The contents of this document do not necessarily reflect the views and policies of the State Water Resources Control Board, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

Table2.4-1. Funding breakdown for this project.

SOURCE			SWRCB	CPB	TOTAL
Personnel Services				$31,233	$31,233
Classification	Hours	Wage/Hour			$24,862
Public Works Director/City Engineer	80	$86.05		$6,884	
Public Works Superintendent	60	$58.65		$720	
Associate Civil Engineer	48	$59.97		$939	
Wastewater System Supervisor	60	$50.92		$1,986	
Engineering Technician	20	$37.49		$188	
Engineering Administrative Secretary	120	$36.15		$4,338	
Lab Analyst/Pretreatment Inspector and Quality Assurance Officer	240	$40.87		$9,809	
Operating Expenses				$3,845	$3,845
Office Supplies, paper, printer ink, label maker, mileage					$674
Professional and Consultant Services				$63,717	
Work plan, QAPP, sampling, lab work, analysis, reporting, subcontracting			$533,672		$533,672
TOTAL REQUESTED			$660,368		
TOTAL INVOICED			$533,672	$98,795	**$559,208**

3. Project Implementation and Reporting Schedule

Item	Table of Items for Review	Date Submitted
	Exhibit A - Scope of Work	
1.	GPS information for Project site and monitoring locations	7/30/2007
2.	Project Assessment and Evaluation Plan (PAEP)	9/5/2007
3.	Monitoring Plan	10/25/2007
4.	Quality Assurance Project Plan (QAPP) and Amendment	10/26/2007 3/13/2008
5.	CEQA/NEPA Documents	08/09/2007
	Work to Be Performed By Grantee	
1.3	Results of Existing Data Analysis Relevant to Fecal Sources at Pismo Beach	10/25/2007
2.1	Contracts with Cal Poly and Environmental Health	4/1/2008 (Cal Poly)
2.2	Sampling and Source Identification Plan and Amendment	10/25/2007 5/16/2008
4.1	Contract with Advanced Liquid Logic	4/23/2008
	Exhibit B. - Invoicing, Budget Detail and Reporting Provisions	
1.	Grant Summary Form	6/3/2008
2.	Quarterly Progress Reports and Invoices	9/10/2008 10/14/2008 1/20/2009 1/20/2010 1/20/2010 4/20/2010 7/20/2010
3.	Annual Progress Summaries	10/20/2008 12/21/2009
4.	Natural Resource Projects Inventory (NRPI) Project Survey Form	Before Final Invoice
5.	Draft Project Report	7/12/2010
6.	Final Project Report	8/11/2010

4. Project Task Description

The tasks involved in this project were broken out into a fecal library collection task, seven different sampling tasks, an ocean current mapping task, four fecal source tracking tasks, an historical data analysis task, a rapid source assay kit development task, a volunteer visual monitoring task and a final data analysis task (Table 4-1).

Table 4-1. Project tasks with start and end dates.

Task	Short Title	Start Date	End Date
1	Fecal Source Library	7/30/2007	5/30/2010
2	Summer Daily Sampling & FIB	7/31/2007	8/25/2008
3	Year Round Sampling & FIB	5/6/2008	5/25/2009
4	Summer Hourly Sampling & FIB	7/16/2008	8/1/2008
5	Rain Event Sampling & FIB	11/4/2008	2/15/2009
6	Pathogen Sampling and Assays	5/6/2008	5/25/2009
7	Ocean Sampling & FIB	6/26/2008	8/25/2008
8	Ocean Current Mapping	7/3/2008	5/25/2009
9	Enterovirus qPCR Assay	7/1/2008	8/1/2010
10	Source Marker PCR Assays	7/1/2008	5/30/2010
11	Multiplexed *Bacteroides* qPCR Assay	not started	not complete
12	TRFLP for Fecal Source Tracking	7/1/2008	5/30/2010
13	Massive Strain Library Ribotyping	5/6/2008	7/29/2010
14	Historical Data Analysis	7/1/2008	7/10/2010
15	Data Analysis and Report Writing	10/30/2008	8/30/2010
16	Rapid Human Source Assay Kit	8/1/2007	6/30/2010
17	Volunteer Beach Survey	5/1/2008	9/1/2008

4.1. Fecal Source Library

Fecal samples from known sources were collected to validate and inform the proposed fecal source tracking (FST) methods. We collected samples from the following sources: sewage, cows, dogs, cats, horses, pelicans, seagulls, ducks and pigeons. Where applicable, at least ten independent samples from separate individuals for each source were collected. We did not collect feces from sea mammals and other birds due to the difficulty of repeat collections and verification of the fecal sources. Samples were tested for Ent, *E.coli* and TC by dilution series and IDEXX assay to retain comparability with FIB counts in water samples and to establish an

average MPN/g of FIB for each fecal source. DNA was extracted to validate and inform the Source Marker and TRFLP FST methods (Tasks 9-12). *E. coli* was isolated for validation of the Ribotyping FST method as part of Task 13.

4.2. Summer Daily Sampling & FIB

This task was designed to support the hypothesis that spring tides play a major role in summer-time bacterial advisory postings. The baseline daily sampling run took place for 30 days in the summer of 2007 starting August 1st and ending August 30th. This sampling took place before the finalized QAPP for the project was in place and the data was used to build a baseline and sampling plan for the following year. The second daily sampling run lasted 60 days in the summer of 2008 beginning June 26th and ending August 25th.

Table 4.2-1. Sampling sites used in all sampling tasks. For maps see Figures 4.2-1 and 4.5-1.

Sampling Site Name	Distance from the beach end of Pismo Pier (m)	Direction	Lon.	Lat.	Frequency/Task
PB1	900	South	-120.63954	35.13109	Week/3, Rain/5
PB2	600	South	-120.64080	35.13358	Week/3, Rain/5
PB3	300	South	-120.64225	35.13605	Day/2, Week/3, Hour/4, Rain/5
PB3.5	150	South	-120.64305	35.13722	Day/2, Week/3, Hour/4, Rain/5
PB3.8	50	South	-120.64374	35.13805	Day/2, Week/3, Hour/4, Rain/5
PB4	12	South	-120.64381	35.13840	Day/2, Week/3, Hour/4, Rain/5, Path/6
PB4.1	12	North	-120.64405	35.13860	Day/2, Week/3, Hour/4, Rain/5
PB4.2	50	North	-120.64429	35.13897	Day/2, Week/3, Hour/4, Rain/5
PB4.5	150	North	-120.64465	35.13986	Day/2, Hour/4, Rain/5
PB5	300	North	-120.64538	35.14108	Day/2, Week/3, Hour/4, Rain/5
L1	500 (lagoon)	SE	-120.63999	35.13540	Week/3, Rain/5, Path/6
C1	Cypress St Bridge	SE	-120.63884	35.13688	Rain/5
C2	Frady Ln Bridge	ENE	-120.63316	35.14285	Rain/5
C3	Ormonde Rd Bridge	NE	-120.62054	35.17794	Rain/5
O4.1	170	Mid Pier	-120.64558	35.13796	Day/2, Rain/5
O4	270	End Pier	-120.64667	35.13766	Ocean/7, Rain/5
O1	4000 (J.O.O.)	South	-120.64505	35.10030	Ocean/7
O2	600 (offshore)	SSE	-120.64398	35.13262	Ocean/7
O3	300 (offshore)	SSE	-120.64551	35.13506	Ocean/7
O5	300 (offshore)	NNW	-120.64847	35.14027	Ocean/7

Sampling sites that cluster around the pier and then extend south along the beach through the area where Pismo Creek forms a lagoon and empties onto the beach at times of high enough flow were selected for the study. Samples were also taken at the mid point (2007 and 2008) and end of the pier (2007) to examine the role of the pier itself as a source of FIB. Ten sites were chosen for daily sampling during 2007 (PB1, PB2, PB3, PB3.5, PB4, PB4.5, PB5, L1, O4.1, and O4). The data collected in the baseline study from summer 2007 was used to improve the choice of

summer 2008 sites. PB1 and PB2 were replaced with new sites closer to the pier where the highest counts are observed. Sampling at L1 for the period 6/26/2008 to 8/26/2008 was changed to a spring tide sampling scheme (section 4.3) since FIB counts were consistently high. The O4 site at the end of the pier was sampled on a separate schedule reserved for ocean sites (section 4.7) based on availability of a boat and captain. The nine 2008 daily sampling sites were PB3, PB3.5, PB3.8 PB4, PB4.1, PB4.2, PB4.5, PB5, O4.1 (Table 4.2-1, Figure 4.2-1). Samples at the beach and lagoon were collected in 30 to 60 centimeters (cm) of water (ankle to knee depth), as is routinely done by SLO-CPHD. A specialized device designed by Cal Poly was used to collect samples off the pier. The summer ocean samples were collected off a boat by scooping up the water while holding the container over the side of the boat at a depth of 30 to 60 cm.

A total of three different samples were collected at each site during these daily sampling runs (2 x 500 mL, 1 x 100 mL). The two 500 mL samples were used for RNA and DNA collection respectively. The 100 mL sample was used for FIB counts via IDEXX, turbidity, salinity and ultraviolet absorbance assays. During summer 2008, an additional 100 mL sample was taken every third day at each site to collect *E. coli* strains for ribotyping (section 4.13). At the full spring tides, additional 15 L samples were collected at PB4 and L1 to test for pathogens (section 4.6). Turbidity, salinity, UV absorbance readings and FIB tests were initiated on the same day as sampling while RNA and DNA samples were filtered, the filtered volume noted (if less than 500 mL) and the filters archived at -80 ºC for nucleic acid extraction later.

Figure 4.2-1. Sampling sites near the Pismo Beach pier.

4.3. Year Round Sampling & FIB

The major sampling efforts took place in the summers of 2007 and 2008 because few FIB limit exceedences were reported for non-summer months (other than near rain events). This smaller sampling effort was necessary to provide a complete picture of fluctuations in FIB over a full year, mirroring the beach monitoring conducted by SLO-CPHD. The sampling dates were picked to be on or as near as possible to the full spring and neap tides to continue collecting data relevant to the tidal cycle. A total of 4 sites (PB3, PB4, PB5, L1) were sampled in the same manner described in section 4.2 above. Three samples were collected every time (2 x 500 mL, 1 x 100 mL). The same tests as for the summer daily samples were performed on this subset of the summer daily samples. At the full spring tides (every other week), an additional 15 L sample was collected at sites PB4 and L1 for pathogen testing (section 4.6). As for section 4.2 above,

turbidity, salinity, UV absorbance readings and FIB culturing and RNA/DNA filtering were performed on the same day as sampling. Filtering and culturing for pathogens also began the same day as sampling (section 4.6).

4.4. Summer Hourly Sampling & FIB

Three hourly sampling runs were performed to determine the effect of the daily tide cycle on FIB counts. The baseline run (24 hours) was performed at spring tide on August 10, 2007 beginning at 4 am and finishing at 3 am on August 11, 2007. This data (section 2.2.2) was used plan the following 48-hr sampling runs. The two 48-hr runs were performed at spring tides in the summer: from 9 am on 7/16/2008 to 8 am on 7/18/2008; and from 9 am 7/30/2008 to 8 am 8/1/2008. Samples (100 mL) from PB3.5, PB3.8, PB4, PB4.1, PB4.2, and PB4.5 were collected every hour to track FIB counts that were performed within 12 hours of sampling. Samples were stored at 4 ºC until processed.

4.5. Rain Event Sampling & FIB

Although AB411 does not mandate sampling of beaches from November through March, it is clear from the SLO-CPHD data that rain events influence beach FIB levels (Figure 2.2.1-1). We expected the sources of FIB to be significantly different during rain events. To test this hypothesis, we sampled 15 sites, PB5, PB4.5, PB4.2, PB4.1, PB4, PB3.8, PB3.5, PB3, PB2, PB1, O4.1, L1 and C1, C2 and C3 during rain events in the wet season of 2008-2009 (Table 4.2-1). The Cypress Street bridge site (C1) does not include input into the lagoon coming from the nearby mobile home park. The Frady Lane bridge site (C2) does not include input from the sewage treatment plant, Highway 101 or the bulk of CPB. The upstream site at the Ormande Road bridge (C3) does not include input from the local homeless camp east of CPB (Figure 4.5-1). The 5 standard samples (section 4.2) were collected at all 15 sites (plus two additional *E. coli* samples – section 4.13) while the 5 pathogen related samples were collected at PB4 and L1 (Tables 1-2, section 4.6). We defined a “rain event” as more than ½ inch of rain reported within 24 hours at the Pismo Chamber of Commerce weather station web site (www.gopismo.com/DavisWeather/Current_Vantage_Pro_Plus.htm).

Figure 4.5-1. Sampling sites located farther away from the Pismo Beach pier (C1, C2, C3 & O1). PB4 and PB1 are also noted (without circles) to orient the viewer.

For each rain event two sampling runs were conducted. The first sampling run took place within 24 hours of the start of the rain event, and the second sampling run took take place within 24 hours of the cessation of rain for a 24 hour period. Four rain events were sampled (two sampling runs each) on the following dates: 11/4/2008, 11/5/2008, 12/15/2008, 12/18/2008, 2/6/2009, 2/1/2009, 2/14/2009, 2/15/2009. As stated in section 4.2 above, turbidity, salinity, UV absorbance readings and FIB tests were initiated on the same day as sampling and RNA and DNA samples were filtered and then archived at -80 ºC for extraction and use later (sections 4.9, 4.10, 4.11). Samples (15 L) were also taken for pathogens (section 4.6). Filtering and culturing also began the same day as sampling.

4.6. Pathogen Sampling and Assays

The main focus of this project was on FIB because they are widely used on a routine basis by regulatory agencies, including the SLO-CPHD, to evaluate water quality standards. The prevalence of certain waterborne pathogens known to cause illnesses was also conducted to supplement our findings on FIB. The rationale of conducting this pathogen task is multi-fold. Some of these pathogens did not show a correlation with FIB counts in previous studies; therefore, their presence in water cannot be accurately estimated based on FIB (Townsend, 1992; Parveen et al., 2008). In addition, when FIB standards were established to indicate the risk of infection, advanced research tools were not readily available to test for multiple, diverse pathogens. Moreover, new and emerging pathogens were not tested thoroughly for any association with FIB. Consequently, traditional FIB counts may inadequately correlate to health risks associated with certain pathogens on beaches (Leclerc et al., 2002; Colford et al., 2007). Therefore, to provide more insight on the microbial quality of Pismo Beach, we chose to monitor a panel of pathogens, listed below, that have a history of causing diseases through exposure to recreational water.

Sampling took place from 5/6/2008 to 5/25/2009. This included 24 samplings during spring tide (Task 3) and 8 samplings during or after 4 rain events (Task 5). The sampling sites were the pier (PB4) and lagoon (L1). We chose PB4 because the risk of infections is likely to be greatest at or near the pier where there are a greater number of visitors. Further, the pier historically has had higher prevalence of FIB in the summer, which indicates the greater likelihood of pathogens present. L1 was chosen because the presence of pathogens in this site would suggest the creek is a major route of transmission. For the following pathogens, standard or conventional detection methods were followed to determine their presence or absence in the samples. In most cases, quantifiable data was also obtained.

4.6.1. *Cryptosporidium* and *Giardia*

Two methods were applied to determine the level of these protozoa. EPA Method 1623 was followed initially. This standard method was designed to test for drinking water but we adopted the method to test for seawater and brackish lagoon water. However, the quality performance standard was not met. Thereafter, a real-time PCR assay was evaluated as an alternative method

but satisfactory results could not be obtained. Consequently, data of these two protozoa provided in the Results section should be regarded as estimation only.

The methodologies are described as follows. In accordance to the EPA Method 1623, at least 10 L of sample was filtered through the FiltaMax Xpress filter module (IDEXX Laboratories Inc., Westbrook, ME) at a flow rate of 1-2 L/minute. The captured oocysts (*Cryptosporidium*) and cysts (*Giardia*) were eluted and resuspended in the elution buffer using the FiltaMax Xpress Elution Station System (IDEXX). After centrifugation at 2000 x g for 15 minutes, supernatant was carefully removed. The pellet was then subjected to immunomagnetic separation (IMS) according to the manufacturer's protocol provided in the Dynabeads®GC-Combo Kit (IDEXX). *Cryptosporidium/Giardia* positive and negative control solutions were obtained from the MeriFlour® *Cryptosporidium/Giardia* Kit (Fisher Scientific, Pittsburg, PA). The amount of oocyst/cyst in the control solutions was verified using direct microscopic count. Following IMS, samples were stained with the reagents provided in the MeriFlour® *Cryptosporidium/Giardia* Kit. Enumeration was carried out using fluorescence microscopy by counting oocyst/cyst that showed the corresponding features according to the EPA website (http://www.epa.gov/microbes/).

4.6.2. *Vibrio parahaemolyticus* and *V. vulnificus*

Methodology was adopted from the Food and Drug Administration's Bacteriological Analytical Manual (FDA BAM). Various volumes (150 mL, 10 mL, 1 mL) of PB4 and L1 samples were filtered through 0.45-µm-pore-size hydrophobic grid membranes (Neogen Corp., Lansing, MI) and regular 0.45-µm-pore-size Nalgene cellulose nitrate membranes (Fisher) for *V. parahaemolyticus* and *V. vulnificus*, respectively. For *V. parahaemolyticus*, membranes were transferred onto Trypticase Soy Agar-Magnesium sulfate-NaCl (TSAMS) plates, incubated at 35°C for 4 h, then transferred to Vibrio Parahaemolyticus Sucrose Agar (VPSA) plates and incubated at 42°C for another 24 h. The number of grids having green colonies was counted. The colonies were streaked onto Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) plate for verification. For *V. vulnificus*, membranes were transferred to TCBS plates and incubated at 35°C for 24-48 h. Putative *V. vulnificus* colonies in dark green were streaked onto Modified Cellobiose-Polymyxin B-Colistin (mCPC) plates. After incubation at 40°C for 24-48 h, yellow colonies on mCPC were counted. An MPN method was also carried out as a supplementary procedure to quantify the amount of these pathogens in the samples. 5-5-5 series of MPN tubes containing Alkaline Peptone Water (APW) was inoculated with 10 mL, 1 mL and 0.1 mL of sample, respectively. Aliquots of tubes showing positive growth were streaked on TCBS and mCPC to confirm the presence of the two *Vibrio* species (dark green colonies on TCBS) and *V. vulnificus* (yellow colonies on mCPC), respectively. *V. parahaemolyticus* FSL-Y1-005 (Yeung et al., 2002) and *V. vulnificus* ATCC 29307 were used as positive controls.

4.6.3. *Aeromonas* spp.

EPA Method 1605, aimed to test *Aeromonas* in drinking water, was adopted for our samples. Various amounts (150 mL, 10 mL, 1 mL) were filtered through 0.45-µm-pore-size Nalgene membranes. Membranes were transferred onto ADA-V plates and incubated at 35°C for 24 h. A small amount (0.1 mL) of L4 sample was also plated onto the same medium. Putative colonies

in yellow were counted and subcultured onto Nutrient Agar (NA) plates. The identity of the colonies was confirmed by a positive oxidase test, the ability to ferment trehalose and to produce indole. *A. hydrophila* ATCC 49140 was used as the positive control.

4.6.4. *Pseudomonas* spp. and *P. aeruginosa*

As most detection methods for *Pseudomonas* apply towards food, beverage and processed water samples, multiple methods were modified to increase our ability to detect *Pseudomonas* spp., especially *P. aeruginosa*, in our samples. First, various amounts (150 mL, 10 mL, 1 mL) were filtered through 0.45-µm-pore-size Nalgene membranes. Membranes were transferred onto King's B plates supplemented with Irgasan and incubated at 35°C for 24 h prior to counting the colonies. Second, 150-mL sample was filtered and the membrane was incubated in King's B broth supplemented with Irgasan at 30°C for 48 h. One milliliter of this enrichment was subjected to an immunoassay following the manufacturer's protocol (TECRA *Pseudomonas* VIA™). Two species of *Pseudomonas* (*P. aeruginosa* and *P. fluorescence*) were used as positive controls. The above two methods were used to detect the presence of *Pseudomonas* spp. For *P. aeruginosa*, samples were filtered as described above. Membranes were transferred onto Modified *Pseudomonas aeruginosa* agar C (M-PA-C) plates and incubated at 42°C for 48-72 h. Colonies of *P. aeruginosa* are 1.0 to 1.5 mm in diameter, flat, dark colored and may have a brownish to greenish-black center. Preliminary PCR assay was carried out according to Tyler et al (1995) to confirm species identity. MPN was also conducted to supplement the membrane filtration. The procedure was similar to *Vibrio* as described above. Tubes of King's B broth supplemented with Irgasan inoculated with the samples were incubated at 35°C for 24 h. Aliquots of tubes showing positive growth were streaked onto M-PA-C plates to confirm the presence of *P. aeruginosa*. *P. aeruginosa* ATCC 10145 was used as the positive control.

4.6.5. *Salmonella* spp.

Methodology was adapted from FDA BAM and Bushon and Koltun (2004). A 150 mL samples were filtered through 0.45-µm-pore-size Nalgene membranes. Membranes were incubated in Selenite Cystine (SC) broth at 35 °C for 48 h. One mL was transferred to 100-mL Rappaport-Vassiliadis (RV) broth and incubated further at 42 °C for 24 hours. Aliquots of the enrichment were streaked onto Bismuth Sulfite (BS) plates and incubated at 35 °C for 24 hours. Identities of these *Salmonella* isolates were confirmed with the LATEX immunoassay according to the manufacturer's protocol (Hardy Diagnostics, Santa Maria, CA). In addition, 5-5-5 series of MPN was also carried out by inoculating SC broth with 10 mL, 1 mL and 0.1 mL of samples, respectively. All tubes were incubated at 35 °C for 48 h. Aliquots of tubes showing positive growth were streaked onto BS and Xylose Lysine Desoxycholate (XLD) plates to confirm the presence of *Salmonella spp*. *S. enterica* ssp. *enterica* (Kauffmann and Edwards), Le Minor and Popoff serovar Typhimurium was used as the positive control.

4.6.6. *Campylobacter* spp.

One to two liters of sample was filtered through 0.45-µm-pore-size Nalgene membranes. During filtration, membranes were periodically rinsed with 100-1,000 mL sterile phosphate buffer to remove excess salt that might inhibit the growth of *Campylobacter*. Membranes were then

placed up side down onto Abeyta-Hunt Bark (AHB) plates, incubated at 35 °C for 24 h in microaerophilic environment. After incubation, membranes were transferred to new AHB plates and incubated for an additional 48 h at 42 °C. Putative colonies of *Campylobacter* (round to irregular with smooth edges, thick translucent white growth, film-like transparent growth) were counted. In addition, 5-5-5 series of MPN was also carried out by inoculating *Campylobacter* enrichment broth containing antibiotics with 10 mL, 1 mL and 0.1 mL of samples, respectively. All tubes were incubated at 35 °C for 24 h microaerophilically. Aliquots of tubes showing positive growth were streaked on AHB plates to confirm the presence of *Campylobacter spp*. *C. jejuni* ATCC 29428 was used as the positive control.

4.6.7. *Shigella* spp.

Methodology was adapted from the FDA BAM and modified to include an MPN method. Various amounts (150 mL, 10 mL, 1 mL) were filtered through 0.45-µm-pore-size Nalgene membranes. Membranes were transferred onto XLD plates and incubated at 35 ^{o}C for 24 h. A small amount (0.1 mL) of L4 sample was also spread plated onto the same medium. For the MPN method, sample was incubated in *Shigella* broth containing novobiocin and incubated at 42 ^{o}C for 20- 24 h in an anaerobic environment. Aliquots of tubes showing positive growth were streaked onto MacConkey plates to confirm the presence of *Shigella spp*. *S. sonnei* ATCC 29930 was used as the positive control.

4.7. Ocean Sampling & FIB

Unless wave fronts move at right angles to the beach, when rip tides are formed, it is common for the action of waves on a beach to form a zone of containment that inhibits transport out of the surf zone into deeper water (Feddersen 1998). This combined with a beach source for FIB could result in the trapping of FIB in the surf zone with a relatively low concentration in the adjacent open ocean. Although we could sample the ocean past the surf zone by using the pier, we could not rule out the possibility that the pier pilings may disrupt normal transport in the water or that the pier may be a source of FIB itself. Thus, we proposed to sample 4 sites out beyond the surf zone during the summer 2008 daily sampling run (Task 2). Sites O2, O3, O4 and O5 are directly off shore of their beach counterparts PB2 through PB5 (Figure 4.2-1). We also sampled directly over the terminus of the Joint Ocean Outflow (site O1) 4 km south of the pier to rule out the outflow as a source of FIB to the beach. Cal Poly's CCMS launched a Zodiac inflatable boat from the Cal Poly pier at Avila to avoid beach boat launches and ensure that the samples could be taken safely under most wave/weather conditions. The 5 standard samples were collected at all 5 sites. Turbidity, salinity, UV absorbance readings, plating for *E. coli* and FIB tests were initiated on the same day as sampling while RNA and DNA samples were filtered and then archived at -80˚C. We only sampled the ocean every third day during the daily sampling run for summer 2008, focusing on spring tide days, since we expected FIB to be very low in the open ocean, an assumption which proved to be correct (Table 6.4.2-1).

4.8. Ocean Current Mapping

The Pismo/Grover/Oceano Joint Ocean Outflow terminates approximately 4 km south of the Pismo Beach pier. Anecdotal evidence suggests that northward near shore currents along Grover and Pismo Beaches are the norm. Although FIB sampling data along the beach near the outflow and south of the Pismo Beach pier do not support the outflow as a source of FIB, knowledge of current flows along the beach and wave height, speed, frequency and direction are important for a complete understanding of the ocean dynamics that may affect FIB counts in the surf zone, and to rule out the outflow as an FIB source for Pismo Beach. To this end, we installed a fixed wave and current sensor, the Nortek AWAC, approximately 50 m off the end of the Pismo Beach pier. The AWAC provided real-time current and wave data to inform sampling and analysis efforts. Specifically, the AWAC provided full water column 3D current profiling and wave period, height, and direction. Because there was a fast response internal pressure sensor for wave period measurements, the AWAC was also used to document the tidal excursions during the study. Cal Poly purchased the instrument in support of this program since its lifetime was estimated to extend well beyond the scope of this project. During the study, CCMS staff provided the CPB and the public with direct real time access to the data for use on surfing and rescue related web pages. This was accomplished by setting up a wireless link between the Pismo Beach pier and the Cal Poly pier in Avila Beach, which is connected directly to servers at Cal Poly. During its deployment, the instrument was inspected twice by divers and the supporting tripod repositioned due to shifting bottom conditions, which were monitored by an internal tilt sensor in the AWAC.

While the AWAC provided excellent detailed real time information, it only covered a single point on the coast. To establish the extent and variability in ocean current patterns along the coast in this area and to assess the potential influence of the Joint Ocean Outflow to the Pismo Beach pier, we employed the use of a REMUS-100 autonomous underwater vehicle (AUV). While traditional techniques (boat-deployed casts and transects) could have provided data for this study, the highly variable coastal system is better sampled with this relatively new tool, which is able to rapidly sample volumes on vertical and horizontal scales on the order of 10 cm. (Blackwell et al. 2007).

The REMUS AUV is fully described in Moline et al. (2005), but will be described here for completeness. Briefly, the REMUS-100 is a propeller-driven platform, which in this application, navigates using a combination of surface GPS, Doppler Velocity Log when in range of the seafloor, and the measured 3D currents surrounding the vehicle. The AUV was instrumented with a 10 Hz Neal-Brown conductivity/temperature sensor for salinity and temperature, a Wetlabs Inc. ECO triplet for measurement of colored dissolved organic material, a Marine Sonics 600kHz side scan sonar, and two 1.2 MHz RD Instruments Workhorse ADCPs, one upward-looking and one downward-looking, on each AUV measuring water velocity relative to the AUV. The two objectives for the REMUS AUV, current mapping and mapping the outfall plume required different combinations of sensors, however were achieved in the same set of missions (see below).

4.9. Enterovirus qPCR Assay

The Enterovirus assay used in this task served two purposes. The first was to check for the presence of human fecal matter in the collected samples. The second was to directly assess the health risk associated with FIB counts in the sampled recreational waters. We developed a quantitative polymerase chain reaction (qPCR) to detect the presence of human-specific enteroviruses. qPCR is a fast and reliable method for microbe detection and allows for high-throughput analysis, none of which are attributes of culture-based methods. However, a complication to this approach is the increased likelihood of false negatives due to co-purification of PCR inhibitors, especially in environmental samples that are known to contain polysaccharides and humic, fulvic, or tannic acids. To address this issue, each of our PCR-based assays (this section and section 4.10) included a competitive internal positive control (CIPC). This is particularly important for the enteroviruses, as there are two enzymatic steps that are subject to inhibition: reverse transcription of the RNA genome to cDNA, and PCR. The amplification of this control is distinguished from the enteroviral target "copy-DNA" (cDNA) by replacing the region complementary to the enteroviral DNA probe with a novel sequence that is complementary to the CIPC probe. The effect of inhibitors on these reactions may be observed as a delay in the environmental sample extract CIPC cycle threshold (C_T) value relative to reactions performed on clean samples.

Samples were filtered and RNA isolated from the filter. Each RNA sample was reverse transcribed into cDNA, which was then used in qPCR analysis on a Cephied SmartCycler. All primers and probes used in the qPCR assay (Table 4.9-1) were based on sequences obtained from Gregory (2006).

Table 4.9-1. Primers and probes used in the Enterovirus qPCR assay.

Primer	Sequence	Target
EV1F	CCCTGAATGCGGCTAAT	PCR of Enterovirus cDNA
EV1R	TGTCACCATAAGCAGCCA	
EV probe	ACGGACACCCAAAGTAGTCGGTTC	Enterovirus - probe site
CIPC probe	TGTGCTGCAAGGCGATTAAGTTGGGT	CIPC- probe site

4.10. Source Marker PCR Assays

We used conventional PCR to qualitatively track the presence of *Bacteroides spp.* associated with humans, dogs, horses and cows: all possible sources of pollution that have host-specific markers (Bernhard, 2000; Kildare, 2007). As described in section 4.9, an internal control (CIPC) was spiked into each PCR reaction to rule out the presence of inhibitors. Amplification of the CIPC was distinguished from the host-specific target based on length of the PCR products (the CIPC being the larger product). Since the impact of any inhibitors cannot be accurately

quantified using this method, normalization based upon the amplification of the CIPC was not possible. Sample DNA from those reactions that demonstrated significant inhibition of the CIPC amplification (based upon gel analysis) were diluted and the assay repeated. It has been observed that this type of inhibition can be relieved in greater than 90% of the samples by simply performing a 2-fold dilution of the extracted DNA (Gregory, 2006).

As positive controls for the Pismo water analysis, we performed serial dilutions of animal specific feces in seawater. Fecal material from humans, dogs, horses or cows weighing 6 g was mixed into a final total volume of 600 mL of seawater. This dilution was serially diluted in ten-fold intervals with seawater. Fecal coliform and *Enterococcus* concentrations were determined for each dilution by standard MPN methods using Colilert and Enterolert by the IDEXX Company. Each dilution series was subject to filtration, DNA extraction and PCR analysis to confirm that seawater did not in any way inhibit PCR amplification.

DNA was extracted from the retentate of 909 filtered Pismo water samples taken from May 2008 to May 2009. These include year round samples (taken weekly), rain event samples and summer daily samples (selected to examine the effect of tide and correlation with FIB counts). Each sample was subject to PCR analysis using host-specific markers from humans, dogs, and horses (Table 4.10-1). A subset of 270 samples (year round and rain events) was analyzed using cow-specific markers.

Table 4.10-1. Primers used in source marker PCR assays

Primer	Sequence	*Bacteroides* Target	Product Size	Reference
Bac32F	AACGCTAGCTACAGGCTT	All (forward)	690	Bernhard and Field, 2000(a)
Bac708R	CAATCGGAGTTCTTCGTG	All (reverse)		Bernhard and Field, 2000(a)
HF134F	GCCGTCTACTCTTGGCC	Human (forward)	590	Bernhard and Field, 2000(b)
BacCan545F	GGAGCGCAGACGGGTTTT	Dog (forward)	150	Kildare et al., 2007
CF128F	CCAACYTTCCCGWTACTC	Cow (forward)	600	Bernhard and Field, 2000(a)
HoF597F	CCAGCCGTAAAATAGTCGG	Horse (forward)	125	Dick et al., 2005
HF190F	GAGTCCGCATGTTCACATG	Human (forward)		this study
HF538R	ATCCTCCGTATTACCGCGG	Human (reverse)		this study

We also had the opportunity to perform PCR analysis on sewage samples obtained from the Pismo Beach and Oceano wastewater treatment plants. The following samples were serially diluted in seawater (10-fold dilution at each step): influent from the Pismo Beach facility,

effluent from Pismo Beach, and the Pismo Beach-Oceano mixed sample that is sent to the diffusers. Each sample and dilution was subject to MPN analysis for *Enterococcus*, total coliforms, and fecal coliforms prior to filtration and PCR analysis using human-specific forward primers.

Every environmental sample that was identified as positive for human-specific *Bacteroides* by PCR, regardless of the intensity of the DNA band that was produced, was subject to further analysis. PCR-positive samples were amplified a second time with the human-specific primer set, yet in the absence of the CIPC. These PCR products were subject to Southern blot analysis using a probe generated from an individual clone from one of the human-specific products (verified by sequence analysis). The probe was designed to not include the primer binding sites used in the PCR assay. This additional test increased the specificity of detection, thereby confirming the presence of human-specific marker. PCR amplification of a dilution series of sewage influent (Pismo Beach wastewater treatment facility) in seawater, as described above, was included on each blot. These samples served as positive controls and allowed a relative quantification of *Bacteroides* in the original water sample, which could be correlated to MPN values that were observed in the sewage samples. (This correlation to MPN values is only valid for untreated sewage samples, as the waste water treatment plant effluent MPN values were found to be more than 1,000 times less than those used in this assay.) We compared the signal density of each positive PCR product to the product generated from the dilution of sewage influent that was found to exceed the AB411 limit in fecal coliforms. This ratio was used to estimate the human influence on any of the samples that exceeded the AB411 MPN limit, if untreated human fecal material was present in the water sample.

4.11. Multiplexed Bacteroides qPCR Assay

The goals for developing a multiplexed *Bacteroides* qPCR assay are two-fold: (1) quantification of amount of source-specific fecal contamination in a given sample and (2) increase the speed and reliability of the analysis.

Prior to developing this assay, we first evaluated the presence of source-specific markers that would be used to quantify the level of contamination in a sample. Conventional PCR was used to amplify *Bacteroides* from DNA samples of a fecal library with the most frequently used (based upon publication record) source-specific primer sets. The variability in signal detection and strength between individual hosts within the same species tested from our fecal library indicated that the development of a qPCR assay would not provide us with a useful means of quantifying source-specific contamination. Individual differences between hosts would undermine any attempt to relate the results to levels of fecal contamination.

The other benefit to using qPCR is improved speed of analysis using a rapid, non-expert kit for fecal source detection. This portion of the project was subcontracted with Advanced Liquid Logics (ALL, section 4.16) and would have included the qPCR assay. Since quantifying source specific contamination was not possible, this part of the project was not pursued.

4.12. TRFLP for Fecal Source Tracking

Because there are a limited number of fecal marker organisms identified at this point, and particularly because there are no marker organisms for bird host species, we investigated the use of Terminal Restriction Fragment Length Polymorphism (TRFLP) analysis (Kitts 2001) in combination with a local fecal library to identify fecal sources from samples taken at Pismo Beach. TRFLP is a method for obtaining a pattern of DNA fragment sizes that correspond roughly to the different types of organisms present in a sample. We used two different sets of PCR primers to collect TRFLP data; a universal 16S rRNA bacterial primer set (Kitts 2001), and the All *Bacteroides* primers set (Table 4.10-1).

DNA was extracted from the fecal library (section 4.1) and TRFLP data collected with both PCR primer sets to determine the capacity of this method to differentiate between fecal sources. In addition, specific fecal sources were used to estimate detection limits for sewage and pigeon feces diluted in seawater. Ten fold serial dilutions of the fecal sources were created with seawater gathered at Pismo Beach from a location with historically low FIB counts (PB2). Samples of each dilution (500 mL) were filtered and DNA was extracted in the same manner as used for the collection of DNA from beach water samples. The resulting TRFLP data was used to determine the concentration of fecal sample that could be reliably detected in a background of the bacteria present naturally in seawater.

We also gathered TRFLP data from summer 2008 samples with very high FIB counts. These results were compared to TRFLP data from the fecal dilution series experiment to see if we could match TRFLP data and thus determine a fecal source for the summer 2008 samples.

4.13. Massive Strain Library Ribotyping

The use of source markers (sections 4.9-4.11) is a rapid and relatively cheap way to identify fecal sources. Unfortunately, specific markers do not exist for many sources and are not specific in other cases (avian sources). The use of a local fecal source library (section 4.12) is also limited in that specific sources may be missed and if the method is untested, detection limits and quantification of source contributions cannot be made clear. Consequently, we included a limited application of an *E. coli* strain library based FST method to balance the possible drawbacks of the other two methods and provide a complete overview of current FST technology. The Institute for Environmental Health (IEH) in Seattle, WA, has the largest source specific strain library in the U.S. (>150,000 strains) and has participated in many studies in California and the Central California Coast. Thus, the IEH is the best choice for participation in the strain library approach to FST. IEH uses a strain fingerprinting method known as ribotyping (Myoda et al. 2003) to match *E. coli* from environmental samples to the strains from known fecal sources residing in their library.

A total of 438 samples, including all rain events (section 4.5) and one third of the summer samples (section 4.2), were used to isolate *E.coli* strains. 100 mL, 50 mL and 10 mL sub-samples from each collected sample were filtered and filters cultured on mFC agar according to the membrane filtration count method #9222 in Standard Methods. Plates with positive *E.coli* colonies were stored at 4 ˚C and shipped on ice to IEH in Seattle for *E. coli* confirmation and ribotyping. In addition, a set of 20 *E. coli* strains (one per fecal sample) was isolated from a

random set of known fecal samples obtained locally (section 4.1) to validate the IEH library with local isolates. Fecal samples were streaked out on MacConkey agar and lactose positive colonies restreaked for purity. Colonies testing positive for indole production and negative for citrate utilization were considered *E. coli* (Myoda et al. 2003) and shipped to IEH in Seattle. Shipments of strains started on 7/2/2008 and continued to 2/17/2009.

4.14. Historical Data Analysis

The SLO-CPHD has collected weekly FIB count data at Pismo Beach since July 2000 and a well-documented set of data from 2005 to 2007 is available for analysis. Statistical analysis of these data was compared to an extensive data set from a Huntington Beach study (Rosenfeld et al., 2006) to determine underlying dynamics that typify bacterial pollution at California beaches as opposed to site-specific issues at Pismo Beach. Data included FIB counts, sampling times and relative tide heights. Analyses included regression models of FIB counts against two tide variables, current tide height and the time since tide was last as high as the current tide.

4.15. Data Analysis and Report Writing

Due to stop work requests and interruptions in funding, original reporting dates were not adhered to (section 3). Periodic informal presentations were provided to the CPB, to the Pismo Beach Ocean Water Quality Committee, a public outreach committee organized by the City and to other public and non-profit organizations (Table 4.15-1). Data analysis goals were laid out for each task and for integrating data across multiple tasks. A general linear model was built to explore correlations between FIB counts and all of the physical and chemical information gathered across the sampling tasks described above (section 6.6.1). Aspects of this model were tested with historical data from Huntington Beach and Pismo Beach (section 6.6.2). Statistical models were also built to explore the relationship between specific source markers (human and dog), FIB counts and the physical and chemical data collected during sampling (section 6.6.3). As mentioned in section 4.12 above, differentiation of fecal sources by TRFLP was determined as well as the detection limit for both sewage and pigeon feces diluted in seawater. These detection limits were compared to FIB counts as well (Section 5.2.3). Statistical models were built to assess correlations between FIB counts and pathogen detection. Correlations of occurrence between pathogens were also assessed (section 6.6.4). And finally, the visual data collected by volunteers (section 6.5) was compared to other observation to bolster conclusions about the origins of fecal contaminants at Pismo Beach (section 7.1.1).

Table 4.15-1. Public Meetings and Presentations

City of Pismo Beach Council Meetings	Ocean Water Quality Committee Meetings	Others
7/17/2007 8/21/2007 4/1/2008 5/6/2008 7/1/2008 10/7/2008 7/7/2009 1/19/2010 6/1/2010 8/17/2010	12/14/2006 1/5/2007 11/8/2007 4/7/2008 7/10/2008 2/19/2009 5/3/2009 5/28/2009 6/16/2009 8/27/2009 11/19/2009 2/4/2010 5/3/2010 7/27/2010	California Water Environment Association Workshop 9/10/2009 Public Workshop 8/11/2010 Surfrider Foundation Presentations 2/23/2008 10/18/2008 4/25/2009

As laid out in the Project Assessment and Evaluation Plan the important biological sources of fecal contamination at Pismo Beach were detailed along with the physical and environmental factors that influence FIB counts (section 7.1). Sets of recommendations were prepared to help determine best management practices at Pismo beach for reducing FIB counts in the future (section 8.1). We also compared the FST methods employed in this study (section 7.2) and offered recommendations for the future use of FST at California beaches (section 8.2). In addition, the incidence and abundance of seven pathogens was analyzed along with associated health risks (section 6.4.4) and some general conclusions made with respect to the dominant fecal source noted at Pismo Beach (section 7.3). Last, we noted the progress made toward development of a rapid portable FST method for detecting human fecal contamination (section 7.4) and noted some future research directions that should help coastal California communities to monitor their beaches (section 8.3).

4.16. Rapid Human Source Assay Kit

The goal of this task was to develop and demonstrate a portable prototype, point-of-sample-collection analyzer including associated sample collection, preparation, and testing apparatus capable of accurately analyzing seawater samples for human specific *Bacteroides*. Quantitative PCR and detection limit studies were conducted using a bench-top prototype of the hand-held device on both *Bacteroides* DNA (on a plasmid produced by EBI) and on raw sewage samples. In the initial assays, ALL attempted to load various dilutions of polluted seawater directly to a digital microfluidics cartridge. For these assays, DNA extraction was performed using either the

Ademtech™ (D-N-Adem™ for Gram Positive and Gram Negative Bacteria) magnetic bead DNA extraction kit or the ChargeSwitch™ (Invitrogen®) beads. The Ademtech™ kit includes a lysis buffer suitable for DNA extraction from bacteria. The parameters for qPCR was optimized on a benchtop instrument using identical primers, times and temperatures compared to assays performed using the on-chip digital microfluidic format. Raw sewage influent, obtained from Pismo Beach and a local wastewater treatment facility, was serially diluted 10-fold to generate 3 concentrations of sample (10^{-1}, 10^{-2} and 10^{-3}). Seawater alone was run in parallel with the serial dilutions as a negative control. The assays targeted the 16S rDNA locus of *Bacteroides spp* with forward primers that were either specific to humans (HF134F) or able to amplify all species within this genus (Bac32F, see Table 4.10-1).

4.17. Volunteer Beach Survey

This task involved the preparation of a volunteer training program, an observation protocol and data sheet and the organization of a group of volunteers to survey the beach during the summer of 2008 when the daily sampling task was in progress. Volunteers took a visual survey of a transect along the beach below the high water mark counting feces inside a 2 meter wide path. They then went up onto the pier and counted the number of people and dogs on the beach and observed behavior of dog owners with respect to picking up droppings.

5. Data Quality Assessment

In compliance with the Data Quality Objectives as listed in the QAPP (Table 7.1), the accuracy, precision, completeness, and detection limits were evaluated on all specified data sets. Representativeness was assured by the sampling design and is not discussed here. Completeness of sampling for all of the parameters specified in the QAPP exceeded 90%, with the exception of the pathogen data sets (section 5.4). We also include a cross-laboratory comparability analysis of FIB data (section 5.3). The pathogen data had several issues with data quality that are discussed in section 5.4.

5.1. Accuracy and Precision

Accuracy and precision were within the parameters specified by the QAPP (Table 7.1) for most of the data collected. The more problematic data sets are discussed below.

5.1.1. Specificity of species specific *Bacteroides* PCR

Primers for the source marker PCR assays (Table 4.10-1) were used to amplify DNA extracted from the fecal library to assess their specificity in detecting *Bacteroides* residing in the fecal material of the of host species examined in this project. The human-, cow-, and horse-specific forward primers only produced a positive PCR result when testing the respective host feces (Table 5.1.1-1). However, some samples of human and cow feces did not produce a PCR product with the host specific primers, even though the universal *Bacteroides* primers may have indicated the presence of *Bacteroides* in the sample. By contrast, the dog-specific primers, while

positively identifying all dog fecal samples, also resulted in PCR products for 7 out of the 10 cat fecal samples tested. This means that we could not differentiate between dog and cat feces in the samples tested. However, the incidence of cat feces in the beach seawater samples collected in this study is most likely negligible. DNA from pigeon feces did not give a positive result with the universal *Bacteroides* primers (data not shown).

Table 5.1.1-1. Specificity of *Bacteroides* PCR

	Feces used to test primers				
Species Specific Primer*	**Human**	**Dog**	**Cow**	**Horse**	**Cat**
Bac32F (all *Bacteroides*)	6/7**	3/3	14/14	18/18	3/3
HF134F (Human)	5/7	0/4	0/14	0/15	0/3
BacCan545F (Dog)	0/7	3/3	0/14	0/18	7/10
CF128F (Cow)	0/7	0/4	13/14	0/15	0/3
HoF597F (Horse)	0/7	0/4	0/14	13/13	0/3

* used in combination with the universal *Bacteroides* reverse primer (Table 4.10-1)
**number of positive PCR results/number of samples tested

5.1.2. Specificity of TRFLP for host species

The ability to differentiate fecal sources by 16S rDNA TRFLP analysis was evaluated using the universal bacterial primers 8dF and K2R (Kitts 2001) and restriction enzyme *Dpn*II. Multi-dimensional scaling (MDS) showed similarities in TRFLP data from feces derived from pet animals such as cats and dogs, grazing animals such as horses and cows, and birds such as seagull, pelican and pigeons (Figure 5.1.2-1). Because this level of differentiation could prove useful, additional dilution series tests were performed to determine the sensitivity of the TRFLP method.

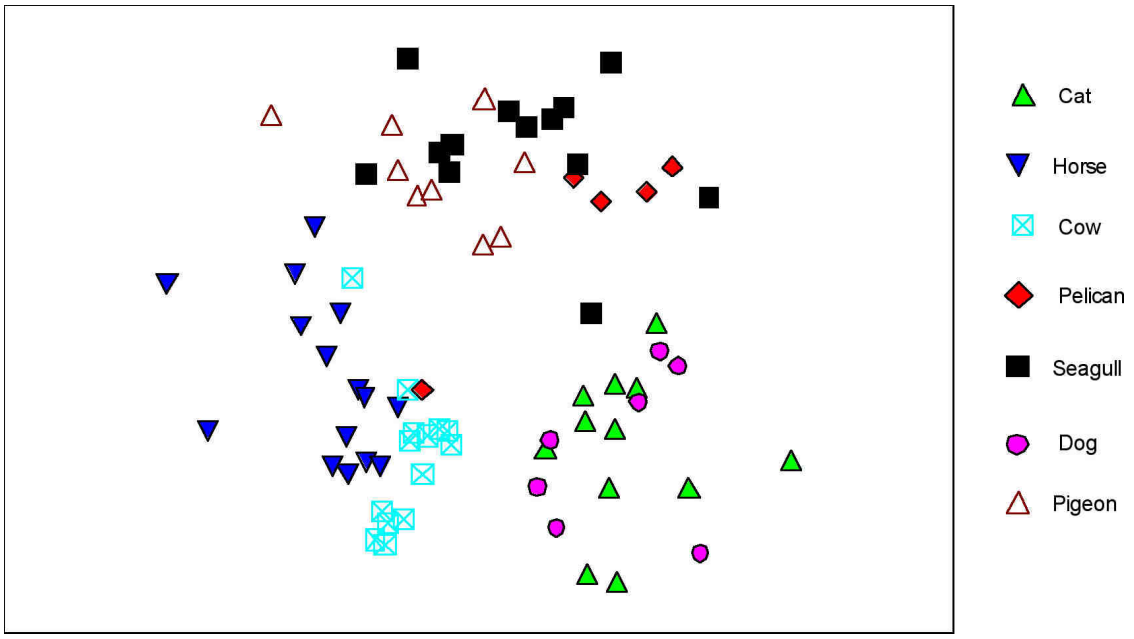


Figure 5.1.2-1. Differentiation of fecal sources by16S rRNA TRFLP.

The universal *Bacteroides* primer set (Table 4.10-1) was also evaluated for use in TRFLP for tracking fecal sources. MDS analysis in conjunction with analysis of similarity for the TRFLP data showed insufficient separation of target species using these PCR primers (Figure 5.1.2-2), so further testing with this primer set was discontinued.

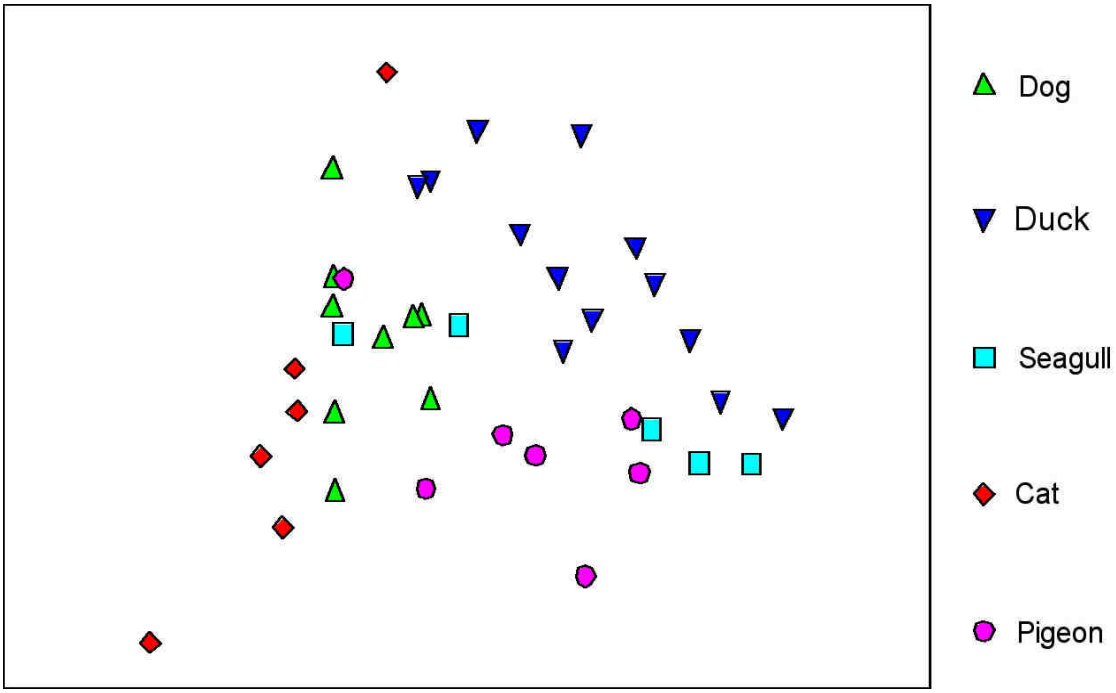


Figure 5.1.2-2. Differentiation of fecal sources universal *Bacteroides* TRFLP.

5.2. Detection Limits

Three methods for source tracking were tested for detection limits to assess their value for tracking sources of fecal contamination at Pismo beach.

5.2.1. Detection limits for *Enterovirus* qPCR

To test the efficacy of the qPCR approach for detecting human enterovirus from fecal contamination in seawater, we analyzed seawater spiked with sewage and poliovirus (a positive control). For comparison, we also analyzed seawater alone, seawater plus poliovirus, distilled water plus poliovirus, and seawater plus sewage (Table 5.2.1-1). An estimated 12,250 polioviruses gave a C_T value of 21.18 while 6125 viruses that had been filtered in distilled water gave a C_T value of 22.43. This C_T value is about one cycle higher than that seen for the control poliovirus sample that was not filtered. This is consistent with an input of half of the 12,250 viruses in the control. From this, we conclude that filtration did not affect efficient recovery of the virus. When 6125 viruses were added to seawater, the C_T value from the cDNA was 23.68. Since this is one cycle higher than that observed for virus filtered with distilled water, it is possible that some component of seawater may have a small inhibitory effect on either retention of virus on the filters, recovery from the filters, or on the qPCR reaction itself. Addition of 0.218 mL of sewage resulted in a slight decrease in the C_T value suggesting that this level of sewage did not contribute inhibitors, or a detectable level of enterovirus. We were also unable to detect any enterovirus from 0.218 mL raw sewage alone. This amount of the same sewage sample contained 20 times the AB411 limit for *E. coli*. These data suggest that detection of human enteroviruses in sewage tainted seawater by qPCR is significantly less sensitive than an MPN analysis for *E. coli* and so this method was not used to analyze the samples collected in the study.

Table 5.2.1-1. Detection of human Enterovirus in sewage and control samples. C_T refers to the number of PCR cycles before the amount of product present crossed a specified threshold.

Sample	Virus Particles	Volume of Sewage	C_T
Seawater	0	0	0
Distilled Water + Poliovirus	6125	0	22.43
Seawater + Poliovirus	6125	0	23.68
Seawater + Sewage	0	0.218 mL	0
Seawater + Sewage+ Poliovirus	6125	0.218 mL	23.06

5.2.2. Detection limits for *Bacteroides* PCR

The amount of fecal source material required to create an AB411 exceedence in 100 mL of seawater was compared to the amount of material detectable in 100 mL of seawater via species-specific *Bacteroides* PCR. Four sources were tested: raw sewage from the Pismo Beach wastewater treatment plant (human), dog feces, horse feces and cow feces. With every source tested, the largest amount of source material was required to produce an AB411 exceedence from a TC count (Table 5.2.2-1). Exceedences from *E. coli* or Ent counts required 10 to 100 fold less source material. The human-specific *Bacteroides* PCR method was able to detect 100 fold less sewage in seawater than was required for an AB411 exceedence with *E. coli* or Ent counts. The same was true for the dog-specific *Bacteroides* PCR method. The cow-specific *Bacteroides* PCR method was the most sensitive, being able to detect 1000 fold less cow feces in seawater than was required for an AB411 exceedence with Ent counts. However, the horse-specific *Bacteroides* PCR method was only able to detect the same amount of feces as would produce an AB411 exceedence from *E. coli* counts. Furthermore, the amount of horse feces required to produce an AB411 exceedence with Ent counts would go undetected using the horse-specific *Bacteroides* PCR method.

Table 5.2.2-1. Detection limits for PCR of *Bacteroides* using human-, dog-, horse-, and cow-specific primers as correlated to AB411 FIB limits (TC – 10,000 MPN/100 mL, *E. coli* – 400 MPN/100mL, Ent – 102 MPN/100 mL).

Fecal Source	Minimum Source Required for an AB411 Exceedence in 100 mL of Seawater			Minimum Source Required for Detection via PCR in 100 mL of Seawater
	TC	*E. coli*	Ent	
Raw Sewage	0.1 mL	0.01 mL	0.01 mL	0.0001 mL
Dog	0.1 g	0.01 g	0.0001 g	0.00001 g
Horse	0.01 g	0.001 g	0.0001 g	0.001 g
Cow	0.1 g	0.01 g	0.001 g	0.000001 g

5.2.3. Detection limits for TRFLP

Detection limits were also assessed for the use of TRFLP to detect sewage and pigeon feces in seawater. In both cases more than 10 to 100 times more source material was required for detection by TRFLP than was required for an AB411 exceedence (data not shown). Consequently, TRFLP was not used for fecal source detection in the rest of the study.

5.3. Comparability

During the EBI's summer of 2008 sampling effort, the SLO-CPHD continued to take weekly samples at PB3, PB4 and PB5 to meet AB411 monitoring mandates for the county. SLO-CPHD agreed to split the samples they collected from late June to late August and split them with the EBI for cross-laboratory comparison. Half of each sample taken was picked up by EBI at the SLO-CPHD labs each Monday morning after they were collected. The split samples were then processed for FIB counts in both labs. The measurements from each lab were consistently within 95% confidence intervals of each other (Table 5.3-1).

Table 5.3-1. Comparison of FIB counts from split samples analyzed by EBI and SLO-CPHD (CPHD). Units are MPN/100 mL.

		TC		*E. coli*		Ent	
Date	**Site**	**CPHD**	**EBI**	**CPHD**	**EBI**	**CPHD**	**EBI**
	PB3	10	20.2	10	10	10	<10
6/30/2008	PB4	52	41.3	20	30.6	10	<10
	PB5	41	30.6	10	20.2	10	<10
	PB3	52	20.2	20	20.2	111	121.1
7/14/2008	PB4	529	271.8	256	84.4	20	<10
	PB5	428	598	223	393.1	10	41.3
	PB3	216	208.6	134	143.5	10	10
7/21/2008	PB4	2723	2909.3	1616	308.6	31	40.9
	PB5	504	454.9	249	173.1	10	<10
	PB3	691	580.6	161	210.9	10	<10
7/28/2008	PB4	884	624.4	272	288.2	10	10
	PB5	110	161.3	20	20.2	42	30.6
	PB3	231	413.5	187	228.1	10	20.2
8/4/2008	PB4	10462	12033.3	6867	10462.4	364	215.7
	PB5	211	312.9	173	278.5	20	74.5
	PB3	201	144.9	85	40.9	10	<10
8/11/2008	PB4	426	331.9	160	132.3	10	<10
	PB5	31	10	20	<10	42	30.4
	PB3	121	107.8	109	63.2	10	10
8/18/2008	PB4	368	335.5	315	299.2	42	62.6
	PB5	272	259	169	171.2	10	<10
	PB3	74	119.9	31	96	20	20.2
8/25/2008	PB4	146	203.4	121	189	10	10
	PB5	41	10	10	<10	10	<10

5.4. Data Quality of Pathogen Assays

5.4.1. Bacterial Assays

For accuracy assessment, positive control organisms were used to determine the quality of each new batch of media (Table 5.4.1-1) and the efficiency of membrane filtration. Negative controls used were either filtered-sterilized samples or sterile saline. All bacterial pathogen assays exceed the initial goal of 90% completeness and 1 per 100 mL sensitivity (Table 5.4.1-2).

Table 5.4.1-1. Quality assessment of microbiological growth media. Batches of media were checked by assessing the growth of control organisms and for expected colony morphology on the agar media indicated (section 4.6).

Medium used	Batches tested	Percent Acceptable	Remarks
ADA-V	26	89%	All confirmed *Aeromonas* isolates showed expected results on this medium and three biochemical tests.
TCBS	26	92%	The control organism failure appeared to be the cause of two questionable batches.
VPSA	23	83%	The control organism failure appeared to be the cause of one to two questionable batches. Samples using questionable VPSA yielded negative to medium levels of *V. parahaemolyticus.*
mCPC	25	100%	This growth medium was always acceptable
M-PA-C	24	96%	Samples using the only questionable batch yielded negative results for *P. aeruginosa*.
AHB	17	53%	Samples using questionable AHB yielded negative or low levels of *Campylobacter*.
XLD	26	89%	Samples using questionable XLD yielded negative to medium levels of *Salmonella* and *Shigella*. Since XLD had some issues in the precision assessment, other assays not relying on XLD were weighted more.
BS	19	100%	This growth medium was always acceptable
MacConkey	26	81%	Samples using questionable MacConkey yielded negative or low levels of *Shigella.*
Campylobacter	23	61%	Samples using questionable Campylobacter broth yielded mostly negative or low levels of *Campylobacter.* Assays using AHB were compared and considered when significant discrepancies were observed in a few incidents.
SC	25	96%	SC and RV were used as duplicate selective enrichment media for *Salmonella*. These media were never questionable on the same date.
RV	25	88%	
King's B + Irgasan	25	100%	This growth medium was always acceptable
Shigella + novobiocin	26	100%	This growth medium was always acceptable

Table 5.4.1-2. Completeness and sensitivity for bacterial pathogen assays.

Pathogen	Completeness	Sensitivity
Vibrio vulnificus	98.4%	1 per 150 mL
Vibrio parahaemolyticus	100%	1 per 150 mL
Aeromonas	100%	1 per 150 mL
Pseudomonas spp	92.2-95.3%	1 per 55.5 mL to 1 per 150 mL depending on assays
P. aeruginosa	95.3-98.4% depending on assays	1 per 55.5 mL to 1 per 150 mL depending on assays
Salmonella	96.9-98.4% depending on assays	1 per 55.5 mL to 1 per 150 mL depending on assays
Campylobacter	98.4%	2 per 100 mL to 1 per 1 L depending on assays
Shigella	98.4%	1 per 55.5 mL to 1 per 150 mL depending on assays

Precision was tested periodically for all bacterial pathogens using relative percent difference (RPD) based on EPA 1605 membrane filtration method for *Aeromonas* (Table 5.4.1-3). As EPA 1605 is designed for testing drinking water, sterile PBS or filtered sample water was used instead of reagent water. Using EPA 1605 method as a guideline, RPD ≤48% is considered satisfactory precision and the number of tests exceeding this level was tracked as a measure of precision throughout the study. Very high precision, as indicated by the percentage of all determinations yielding RPD ≤48%, was achieved for *Pseudomonas aeruginosa* on *M*-PA-C (100%) and *Aeromonas hydrophila* on ADA-V (85%). Other pathogens yielded acceptable precision, such as *Vibrio* spp on TCBS (~70%). The XLD medium presented a continuous problem. However, multiple methods were used to determine the presence of the relevant pathogens, and method(s) not relying on XLD medium carried more weight in the resulting analyses.

Table 5.4.1-3. Precision measurements using control bacteria on specific growth media. Precision is measured as the percent of tests with RPD ≤48%. All tests used spiked filter-sterilized PB4 sample water or sterile deionized water. Some analyses were performed in duplicate as indicated (dup).

Pathogen	Medium	RPD tests	Precision	Remarks
V. parahaemolyticus	VPSA	10	50%	Actual sample testing did not involve a filter membrane immediately placed on VPSA. A recovery step was performed prior to using VPSA.
V. parahaemolyticus	TCBS	11	73%	Actual sample testing did not directly test *V. parahaemolyticus* on membrane placed on TCBS. The purpose of this test was to compare the precision of testing for *V. vulnificus* on TCBS
V. vulnificus	TCBS	12 (dup)	67%	Good precision.
V. vulnificus	mCPC	11	45%	Actual sample testing did not involve a membrane placed immediately on mCPC. Putative *V. vulnificus* isolates on TCBS were streaked out.
S. Typhimurium	XLD	11	18%	Actual sample testing did not directly test *Salmonella* on membrane placed on XLD. The purpose of this test is to compare the precision of *Shigella* on XLD.
S. Typhimurium	BS	11	45%	Actual sample testing did not involve membrane placed on BS. Two rounds of selective enrichment were carried out prior to streaking isolates on BS.
Shigella sonnei	XLD	12 (dup)	33%	Since precision was relatively low, more weight was put on the MPN assay for quantifying *Shigella*.
P. aeruginosa	*M*-PA-C	11	100%	Excellent precision.
Aeromonas hydrophila	ADA-V	13 (dup)	85%	Excellent precision.
Campylobacter jejuni	AHB	9	56%	Membrane filtration methodology for *Campylobacter* was more appropriate to determine presence/absence of pathogen.

5.4.2. Protozoan Assays

Data quality objectives were not reached with assays for quantifying *Cryptosporidium* and *Giardia*. The percent recovery of parasites was well below that recommended by EPA Method 1623. Duplicate analyses of parasite assays were performed on three samples but all resulted in low percent recoveries (often zero)**.** While recoveries showed improvement following troubleshooting and consultation with the QA Officer, less than 50% of control experiments met EPA standards. A method modification aimed at better dissociation of the parasites from

immunomagnetic beads appeared to help increase the percent recovery but results were still inconsistent. Many L1 samples clogged the filters, making downstream processing difficult if not impossible. Since EPA method 1623 is intended for drinking water samples, it is not surprising that this method was not easily adapted to our samples due to high concentrations of particulates. As a result, <50% of the samples yielded quantitative results using EPA 1623. Despite a lack of good quality quantitative data, qualitative results are presented in Section 6.4.5.

5.5. Blind test of IEH Fecal Source Library

To test the accuracy of massive *E. coli* strain library ribotyping (section 4.13) as an FST method we sent a random set of 20 *E. coli* strains to IEH which had been isolated from known fecal samples in our local fecal library (section 4.1). There were 3 *E. coli* strains from cats, 1 from a cow, 6 from dogs, 2 from ducks, 1 from a horse, 5 from pigeons and 2 from gulls. IEH was not informed of their origins until after their results were returned to us. Two strains we sent to IEH did not produce a usable ribotype. Out of the 18 remaining strains tested, only three produced a match to ribotypes already held in the IEH library, a remarkably low result. One strain we isolated from a dog matched a dog-isolated strain in the IEH library. Another dog-isolated strain matched a strain isolated from an avian source in the IEH library, while a cat-isolated strain matched a strain in the IEH library isolated from a dog.

These results were discussed with IEH and the following explanations were offered. Every animal harbors a range of *E. coli* strains, many of which are not specific to the host (transient strains) and may be seen in other hosts. In addition, many strains do not survive well in the environment and so are not often found in water samples. Consequently, when isolating *E. coli* from feces there is a high probability for isolating unique strains (not already in the IEH library) and transient strains (which are purged from the IEH library when they are discovered). We cannot determine if the return of one good match from 18 isolates is a normal result without further experimentation.

6. Results

6.1. Site Conditions throughout Sampling

Pismo Beach on the Central Coast of California exhibits a Mediterranean climate with temperature extremes buffered by the cold Pacific Ocean. Highs range from the rare summer day reaching 40 ^{o}C to a rare cold winter day of 5 ^{o}C. Lows in summer can dip to 10 ^{o}C as the fog comes in, and it may occasionally reach freezing on a rare winter night. Rainfall in the Pismo area averages at 10 to 15 inches per year, most falling between November and May. The year before sampling for this study began in earnest, 2007, was a very low rainfall year. More rain fell during the 2008-09 wet season, but the total was still below average.

Other events that may affect microbiological conditions in the ocean include the close passage of “bait-balls”, large congregations of anchovies or other small fish that will sometimes congregate near the surface along the coast during upwellings. These bait-balls attract large numbers of

marine mammals and seabirds whose fecal material may have an impact on beach water quality. Two such events were noted by volunteers during the summer 2008 samplings.

6.2. Oceanographic Results

Oceanographic results are divided into three sections. First, an assessment of the Pismo/Grover/Oceano Joint Outflow influence on local conditions was made using data obtained from the REMUS AUV. Secondly, an area survey of the currents in and around the Pismo Beach pier and AWAC sensor was made by the REMUS AUV to illustrate that the measurements of waves and currents made by the AWAC are representative of the surrounding area and can be applied to the sampling grid (Figure 4.2-1). Lastly, an analysis of the wave and current data from the AWAC was made to provide a context for the water sampling and results from those samples in this study.

6.2.1. Monitoring Pismo/Grover/Oceano Joint Outflow

On 7/3/2008, 7/31/2008, 8/14/2008, the REMUS AUV was deployed from the Cal Poly pier, traversing to the area of the Pismo/Grover/Oceano Joint Outfall (Figure 6.2.1-1). Here, the vehicle first conducted a fine resolution grid at a fixed altitude off the bottom for a side scan sonar mission of the outfall to ensure positioning of the water column mapping (Figure 6.2.1-2). After the side scan sonar mission was completed, the vehicle conducted both an east-west grid and an overlapping north-south grid covering a 1.2 km box around the outfall. The vehicle was undulating while conducting these grids, so the full volume around the outfall was characterized for salinity and temperature as well as colored dissolved organic material (cDOM). The combination of low salinity waters and cDOM are excellent markers for effluent from the outfall. Here, we used these parameters to identify effluent and estimate dilutions.

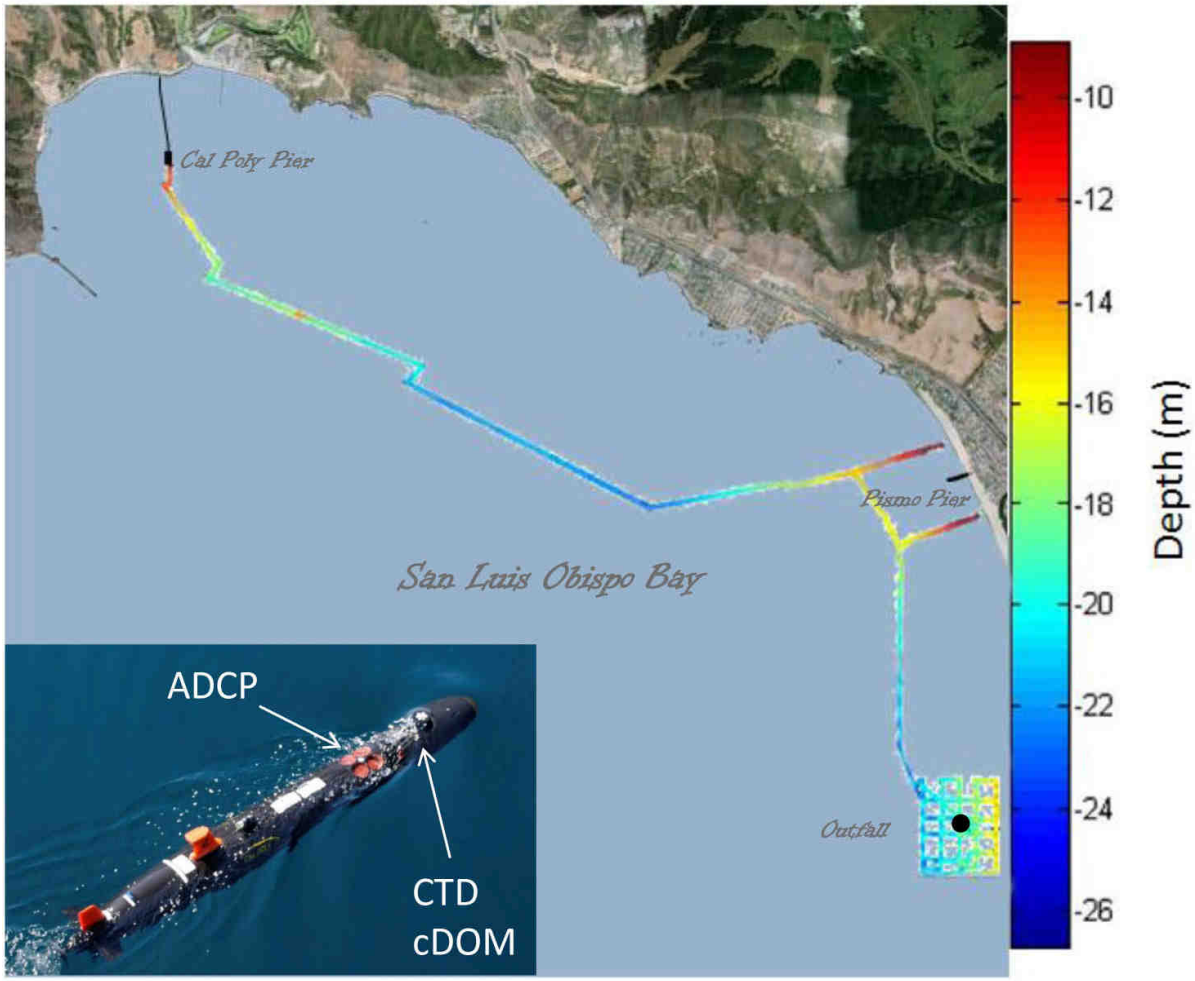


Figure 6.2.1-1. Map of the sampling area conducted by the REMUS AUV. The vehicle (inset) was launched and recovered from the Cal Poly pier. It first conducted the survey over the Pismo/Grover/Oceano Joint Outfall and then moved in shore for two transects on either side of the Pismo Beach pier for a cross-shore assessment of the currents. The color overlay on the vehicle route shows the bathymetry from the 7/3/2008 mission. Labels on the inset indicate the position of sensors on the AUV for detecting temperature (ADCP), salinity (CTD) and cDOM.

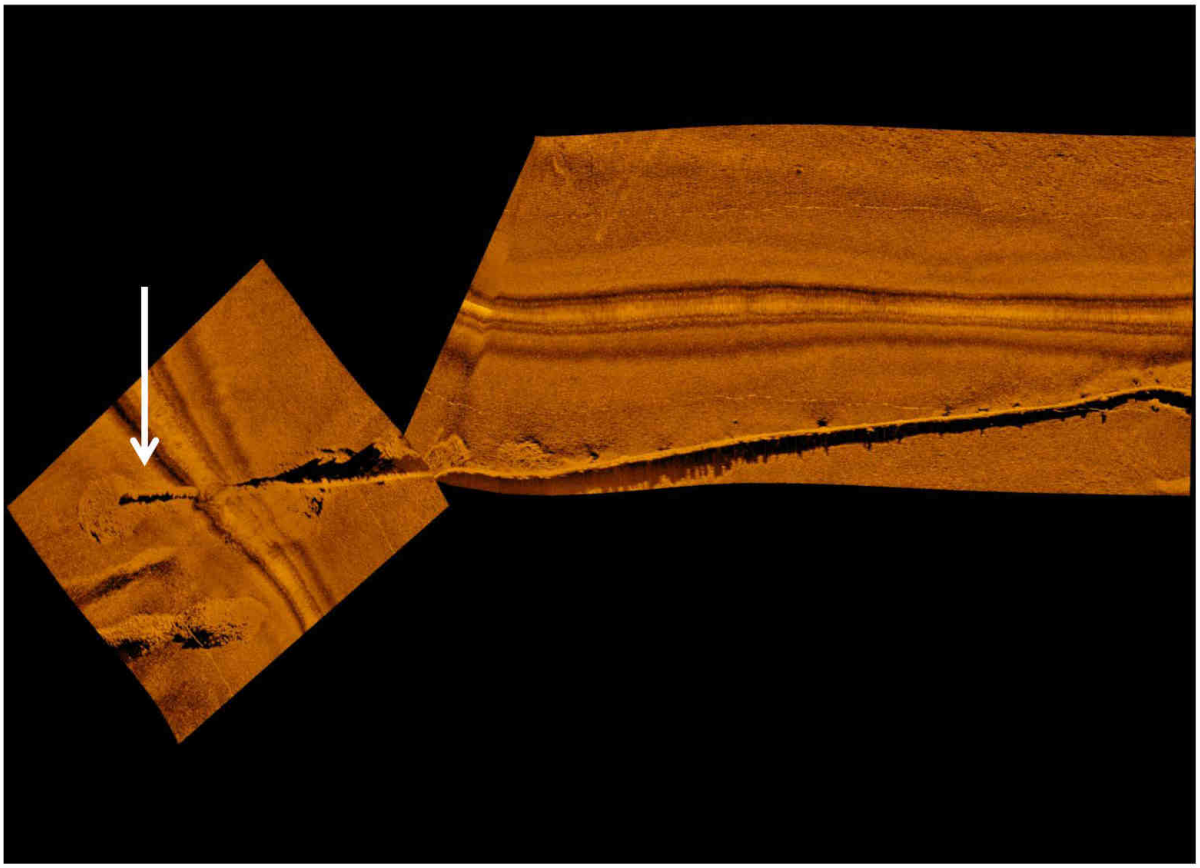


Figure 6.2.1-2. Side scan sonar image of the Pismo/Grover/Oceano Joint Outfall (terminus indicated with a white arrow) conducted on 7/3/2008.

From the depth distribution of cDOM during the three deployments, it is clear that there are consistent sources of cDOM in the mid-water depth from 10-14 m (Figure 6.2.1-3). These elevated signals are consistent with lower salinity water and identify the outfall effluent. If these data are visualized as a function of distance from the outfall, the effluent is not found beyond 600 m from the source, with the most pronounced signals consistently within 100 m of the outfall. Although the direction of the effluent changed over the three missions, there was a general flow of these layers to the northeast.

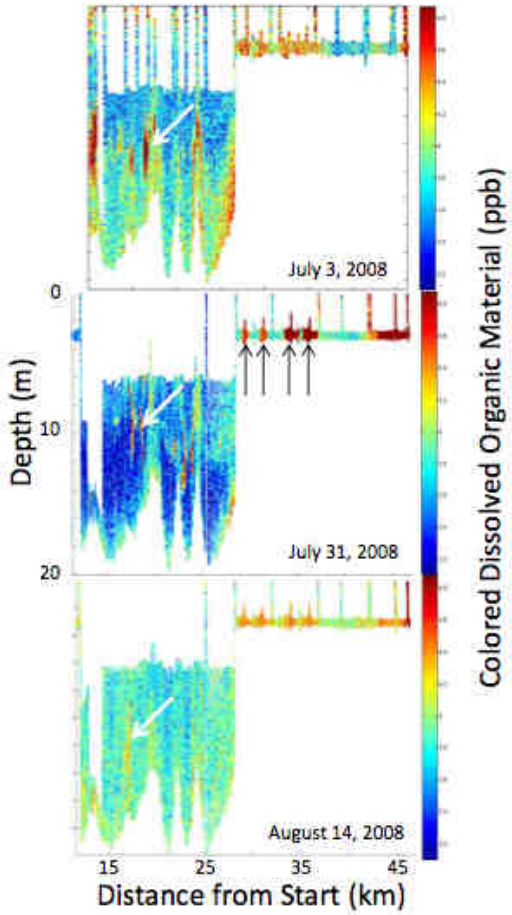


Figure 6.2.1-3. Depth distribution of cDOM in the water column as a function of distance along the route (Figure 6.2.1-3). The portion of the figure prior to 24th km is the grid in and around the outfall, with the following portion representing transects along the Pismo Beach pier (black arrows) and the return to the Cal Poly pier. Within the volume around the outfall, there are clear increases in cDOM at about 10-14 m (white arrows) on all three sampling days identifying the effluent from the outfall.

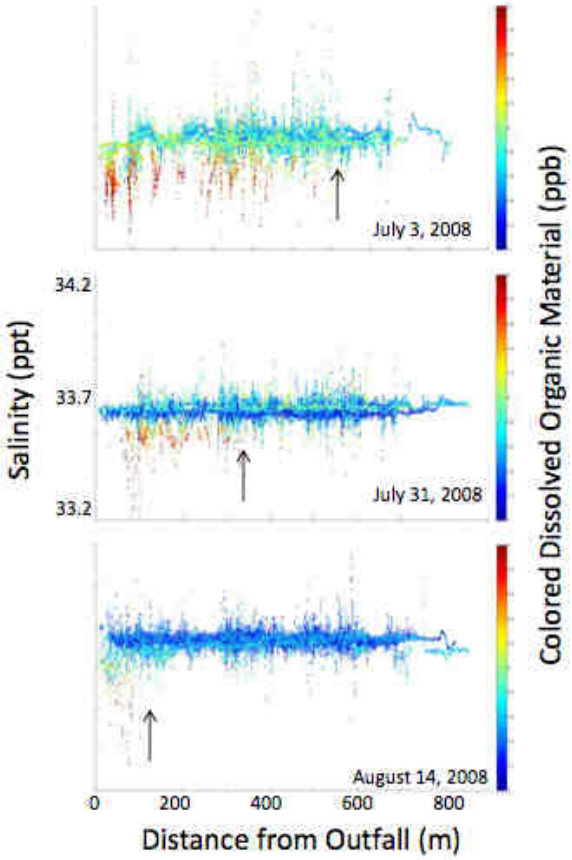


Figure 6.2.1-4. Salinity as a function of distance away from the Pismo/Grover/Oceano Joint Outfall for each of the three missions. Overlaid on the salinity values are the concurrent values for cDOM. Effluent from the outfall is characterized by both lower salinity and high cDOM. Black arrows indicate the distance at which effluent was no longer detected.

Because the effluent plume is mainly fresh water, its density is less than the saltier ocean waters and it is driven upward to the surface by buoyancy forces. As the plume rises, its salinity steadily increases as it mixes with ambient ocean waters. Water parcels consisting of mixtures of effluent and ocean waters may be identified by their lower salinity compared with background ocean waters. These waters mixed and stratified at depths of 10-14 m and were distributed around the source (Figures 6.2.1-3 and 6.2.1-4). The salinity difference between the background ocean salinity (S_b) and the measured salinity of a mixture of effluent and ocean water (S_m) is related to the dilution D according to the equation $D = (S_e-S_b)/(S_m-S_b) = S_b/\Delta S$ where S_e is the salinity of the effluent (assumed to be 0) and $\Delta S = S_b-S_m$. For this study, the upper end salinities for each transect (Figure 6.2.1-4) approximate S_b in each case.

To reduce the likelihood of errors in estimating a cut off dilution value, two approaches were used to estimate typical dilutions for the three REMUS missions, following procedures detailed in Ohlmann et al. (2010). In the first approach, dilution values (D) were computed at the four stations for each sampling event only when $\Delta S \geq 0.05$. This limits the maximum detectable

dilution to about 600, but reduces errors in ΔS resulting from natural variability in S_m and S_b. In the second approach, values of D were computed for $\Delta S \geq 0$ which allows higher values of D, although some of the highest may result from natural salinity variability and therefore be erroneous. Median dilution values from the first approach (D_L) are interpreted as lower bounds on typical dilutions at the four stations and median dilution values from the second approach (D_U) are interpreted as upper bounds. Median rather than average values for D are used since average values are more affected by outliers.

A minimum dilution of D = 99 for the entire sampling period was recorded within 100 m of the diffuser on 7/3/2008 (Table 6.2.1-1). This value for a near-field dilution of 100 is a typical design criterion for ocean outfalls (Fischer et al., 1979). Median dilutions at the diffuser were 300 to 849, with a rapid increase to > 450 at a distance of > 100 m. The lower and upper dilution estimates (D_L and D_U respectively) are given in columns 3 and 6, respectively. The percentages of ΔS values exceeding 0.05 (column 4) and exceeding 0 (column 7) steadily decrease with distance from the diffuser, similar to results reported by Ohlmann et al. (2010). Thus, within half a kilometer from the diffuser the least dilute effluent water measured was 244, while most often the dilution ranged from 500 to 4000.

Table 6.2.1-1. Summary of dilution estimates (see text for explanation).

Outfall Distance (m)	Min D	D_L	% ΔS ≥ 0.05	D_U	% ΔS ≥ 0	n
0 - 100	99	300	37	849	90	6073
100 - 200	181	502	12	3184	46	3017
200 - 300	176	465	6	4204	35	3020
300 - 400	257	484	7	2294	46	4822
400 - 500	121	463	5	3680	41	5724
500 - 600	251	512	2	4034	21	12284
600 - 700	244	506	5	3244	47	3177

6.2.2. Spatial Distribution of Currents off Pismo Beach

For the second objective of the three REMUS AUV missions, the vehicle transited from the Pismo/Grover/Oceano Joint Outfall towards the Pismo Beach pier (Figure 6.2.1-1). Here, the vehicle conducted at least two back and forth transects to the south and then north of the pier at a fixed depth of 2.5 m to resolve the along- and cross-shore currents. The objectives of this phase of each mission were: 1) to evaluate whether there was cross-shore variability in currents and/or 2) to determine whether there was a difference between the north and south transects around the pier. Both of these objectives were implemented to ensure that the AWAC sensor deployed at the end of the Pismo Beach pier was delivering data representative of the currents not only at the end of the pier, but along both sides of the pier and further offshore.

Results from the REMUS AUV indicated minimal currents on all three deployments. Figure 6.2.2-1 shows the east and north components along the southern transect on 8/14/2008.

Velocities were on the order of 5-15 cm/second to the NNE, with an offshore flow in the surface waters above depths of approximately 7 m and a slight onshore flow below that. The repeat transects also showed consistency over the 45 minutes it required to conduct the southern transects. For comparison, the AWAC data showed similar flows for the week around the REMUS sampling time (Figure 6.2.2.-2), with much of the temporal variability in the two components of the currents tidally driven.

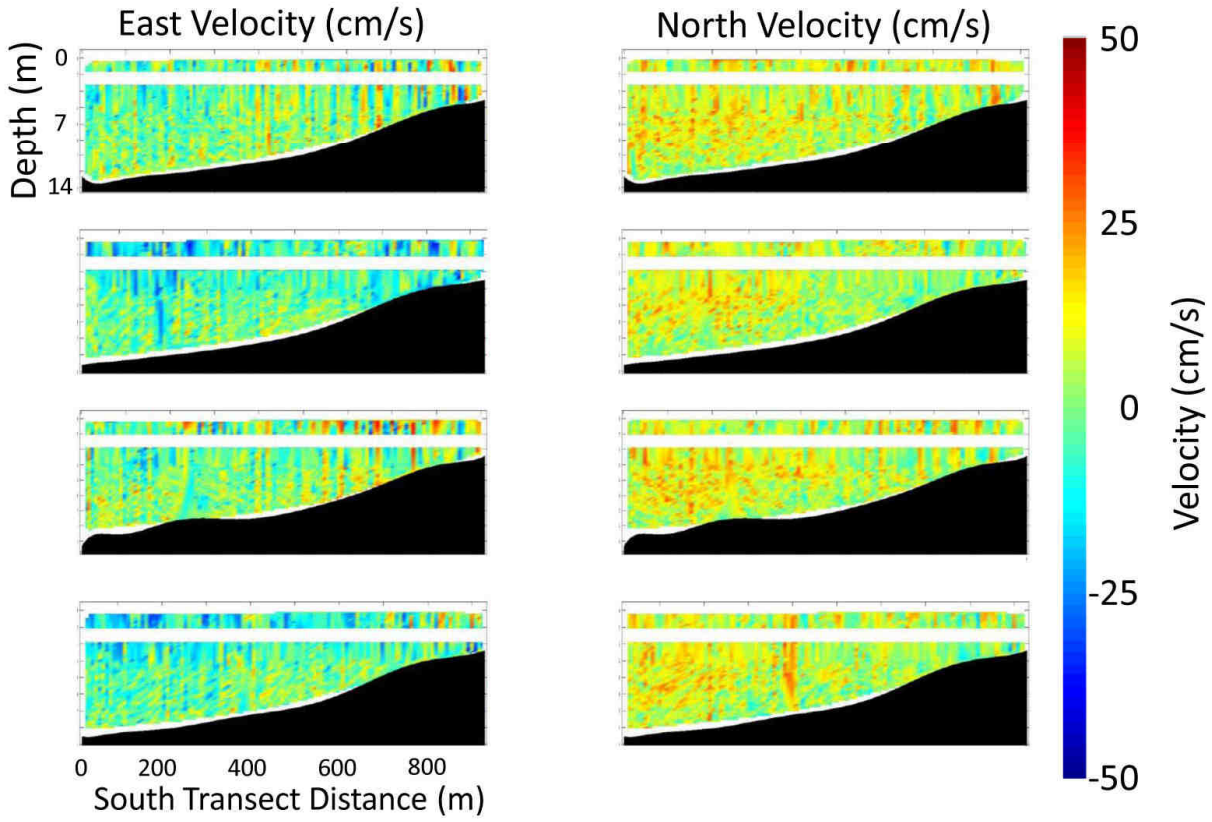


Figure 6.2.2-1. Cross-shore transects of the east and north components of the water column currents along the southern transect (see Figure 6.2.1-1.) on 8/14/2008. The distances of the four transects are from the offshore beginning of each transect. The contour of the bottom is in black.

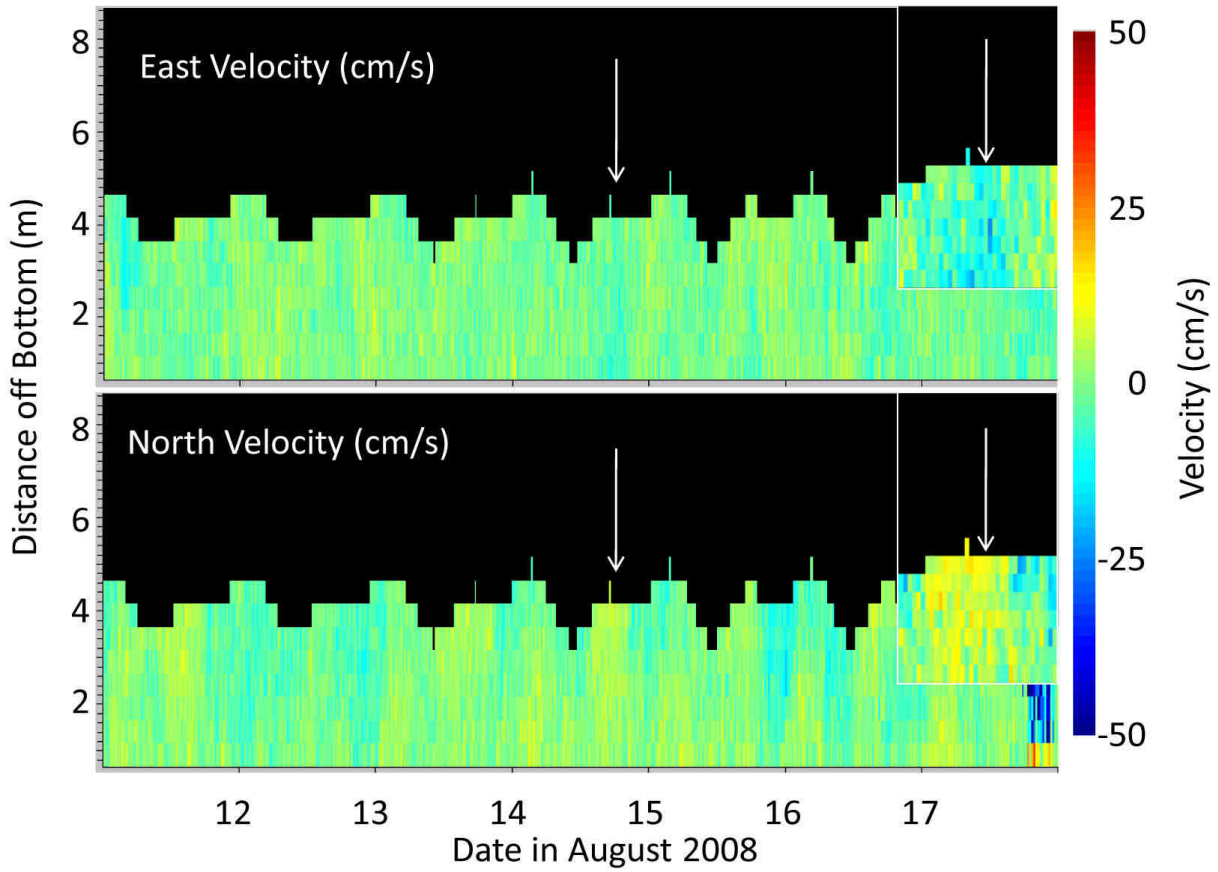


Figure 6.2.2-2. Example of the east and north components of the water column current measured from the AWAC during the week of 8/11/2008. The white arrow indicates the time that the REMUS was conducting its transects (Figure 6.2.2-1.). The inset on the right of each panel shows a blow up of this sampling period.

In directly comparing the currents derived from the REMUS and AWAC during the three missions, it was clear that the two platforms were measuring within 2 cm/s of each other for each component , with similar variability (Table 6.2.2-1). In the context of this study, the current data from the AWAC were a good representation of the entire area around the Pismo Beach pier and along the shoreline. This provides confidence in attempting to interpret results from the water sampling in the context of the oceanographic dynamics.

Table 6.2.2-1. Comparison of current velocities measured by the AWAC and those measured by the REMUS AUV ADCP. AWAC data for the water column was time averaged during the REMUS mission in the area, while the REMUS data was spatial median for the two transects along the Pismo Beach pier (Figure 6.1.1-1.), "±" refers to the standard deviation.

Date	AWAC Velocity (cm/s)				REMUS Velocity (cm/s)			
	North		East		North		East	
	$\tilde{x}$	±	$\tilde{x}$	±	$\tilde{x}$	±	$\tilde{x}$	±
July 3, 2008	4.20	35.00	-0.90	34.00	3.77	16.75	-0.36	23.23
July 31, 2008	0.80	2.90	-2.50	3.00	-0.39	17.44	-2.50	6.01
August 14, 2008	2.70	3.00	-3.20	4.20	3.00	17.96	-2.37	6.14

6.2.3. Analysis of Waves and Currents off Pismo Beach

The AWAC instrument purchased by Cal Poly was placed on the ocean bottom, 50 m off the Pismo Beach pier and collected data during the entire sampling regime from 5/24/2008 to 5/25/2009. Data was collected on wave energy and direction, depth and water currents from 1 m above the bottom up to the surface in 0.5 meter increments. Because the data was transmitted to a storage computer at small intervals, there is a massive amount of information available in this collection (http://marine.calpoly.edu/researchprograms/pismo.php). Consequently, the data was averaged over 1 hour intervals to match with sampling times at each site along the beach and five variables (Table 6.2.3-1) were used in later analyses to look for effects on FIB counts. Variables were named as follows: Hm0 = significant wave height (meters); Tm02 = Mean wave period (seconds); Mdir = Mean wave direction (degrees from north) a weighted average of all directions of the wave spectrum - weighted according to the energy at each frequency; Cur = onshore current (CurX) and alongshore current (CurY) in meters/second.

Table 6.2.3-1. Summary statistics of AWAC data used for analysis later in the study. Data is averaged by sample time at each site and presented as Mean±Standard Deviation (N).

Site	Hm0	Tm02	Mdir	CurX	CurY
PB1	1.15±0.40 (7)	5.46±0.87 (7)	248±7.6 (7)	0.03±0.14 (7)	0.00±0.11 (7)
PB2	1.15±0.41 (7)	5.42±0.83 (7)	247±7.5 (7)	0.04±0.14 (7)	0.00±0.11 (7)
PB3	0.770±0.24 (90)	5.50±0.85 (90)	248±4.8 (90)	-0.01±0.05 (90)	0.03±0.07 (90)
PB3.5	0.766±0.18 (158)	5.00±0.77 (158)	249±3.8 (158)	0.00±0.04 (158)	0.03±0.06 (158)
PB3.8	0.766±0.18 (157)	4.99±0.77 (158)	249±3.9 (157)	0.00±0.04 (157)	0.03±0.04 (157)
PB4	0.773±0.18 (180)	5.13±0.88 (180)	249±4.0 (180)	0.00±0.05 (180)	0.03±0.04 (180)
PB4.1	0.774±0.19 (158)	5.00±0.77 (158)	249±4.0 (158)	0.00±0.05 (158)	0.03±0.06 (158)
PB4.2	0.774±0.19 (159)	5.01±0.77 (159)	249±4.0 (159)	0.00±0.05 (159)	0.03±0.06 (159)
PB4.5	0.775±0.19 (159)	5.03±0.79 (159)	249±3.9 (159)	0.00±0.05 (159)	0.03±0.06 (159)
PB5	0.788±0.25 (91)	5.58±0.80 (91)	248±4.8 (91)	0.01±0.06 (91)	0.03±0.08 (91)
O1	0.705±0.16 (20)	5.34±0.71 (20)	248±3.3 (20)	-0.02±0.03 (20)	0.02±0.03 (20)
O2	0.703±0.16 (20)	5.34±0.71 (20)	248±3.3 (20)	-0.02±0.03 (20)	0.02±0.03 (20)
O3	0.703±0.16 (20)	5.34±0.71 (20)	248±3.3 (20)	-0.02±0.03 (20)	0.02±0.03 (20)
O4	0.704±0.16 (20)	5.33±0.71 (20)	248±3.3 (20)	-0.02±0.03 (20)	0.02±0.03 (20)
O4.1	0.759±0.25 (63)	5.34±0.63 (63)	248±4.9 (63)	0.00±0.06 (63)	0.03±0.09 (63)
O5	0.705±0.16 (20)	5.36±0.69 (20)	248±3.4 (20)	-0.01±0.04 (20)	0.026±0.03 (20)

Wave direction and current data were also analyzed with respect to the times at which samples were taken and graphed to show distributions during sampling events (Figure 6.2.3-1). Five sampling times coincided with anomalously large currents, either offshore or down the shore. These actually correspond to two separate sampling days, with sampling times falling in adjacent one-hour bins. The distribution of wave directions focused around 245 to 250 degrees with most samples taken when waves were coming from slightly north of the pier since a 244 degree angle is perpendicular to the beach.

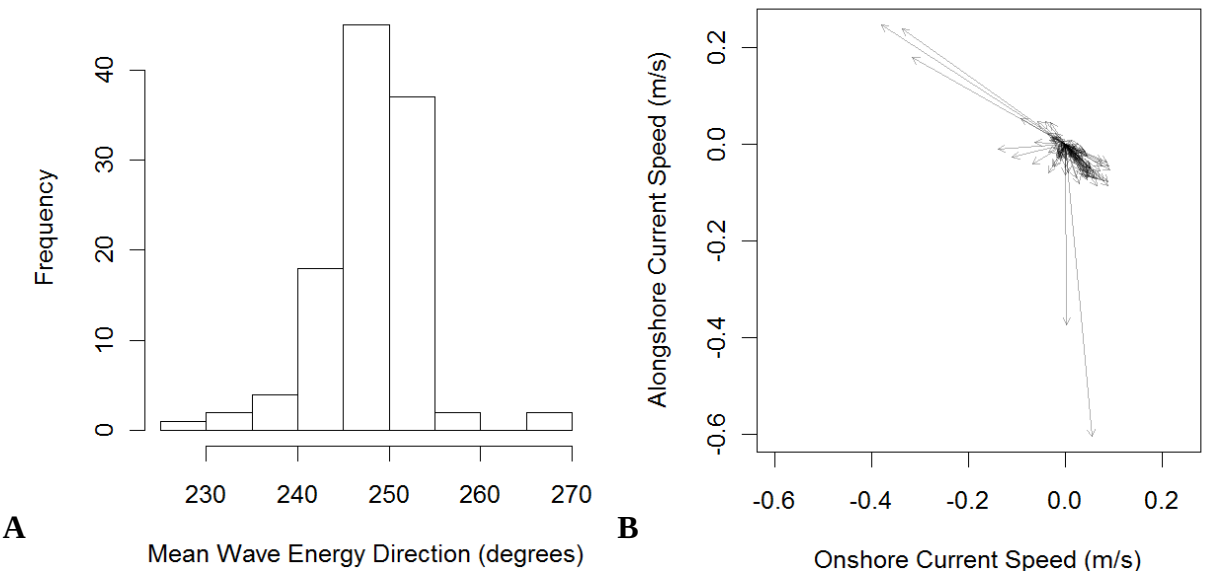


Figure 6.2.3-1. Wave direction (A) and current speed and direction (B) data collected only when FIB samples were taken. An angle of approximately 244 degrees is perpendicular to the shore at the pier. Onshore current is positive when water is moving toward the beach. Alongshore current is positive when water is moving northwards along the angle of the beach.

6.3. Physical and Chemical Results

A set of 7 physical and chemical parameters were measured throughout the sampling period from 5/6/2008 to 5/25/2009 and are presented here as averages by site (Table 6.3-1). Not all variables were collected for every sampling scheme. For example, wind velocity and direction were not collected during the hourly sampling runs (see section 4). Variables were named as follows: Salinity (millisiemens/centimeter); Turbidity (Nephilometric Turbidity Units); UV254 = absorbance of ultraviolet light at a wavelength of 254 nm (absorbance); Rain = precipitation per day (inches/day); MSL = mean sea level relative to low water (feet); Wash = calculated time since the tide was last as high as present (hours); Wind = onshore wind speed (WindX) and along shore (WindY) in meters/second.

Table 6.3-1. Summary statistics of all physical and chemical data broken out by sampling site. Data is represented as Mean± Standard Deviation (N). The number of measurements (N) varies because some measurements were not taken during all sampling runs.

Site	N	Salinity	Turbidity	UV254	Rain	MSL	Wash	WindX	WindY
C3	8	1.01±0.587 (8)	20.6±18.9 (8)	0.429±0.140 (8)	0.095±0.144 (8)	0.637±0.456 (6)	6.83±8.59 (6)	-0.21±2.9 (7)	-0.74±2.12 (7)
C2	8	1.58±1.07 (8)	31.5±49.1 (8)	0.359±0.205 (8)	0.095±0.144 (8)	0.557±0.492 (6)	7.83±7.73 (6)	-0.21±2.9 (7)	-0.74±2.12 (7)
C1	8	17.4±5.72 (8)	14.4±9.09 (8)	0.388±0.166 (8)	0.095±0.144 (8)	0.878±1.13 (6)	7.5±7.87 (6)	-0.21±2.9 (7)	-0.74±2.12 (7)
L1	48	19.8±12.6 (44)	9.19±11.3 (44)	0.386±0.376 (44)	0.024±0.075 (48)	0.184±1.87 (38)	8.45±18.5 (38)	-0.52±1.87 (25)	-0.33±1.47 (25)
PB1	8	48.3±3.18 (8)	4.21±2.16 (8)	0.020±0.012 (8)	0.095±0.144 (8)	0.929±1.15 (7)	4.29±3.15 (7)	-0.21±2.9 (7)	-0.74±2.12 (7)
PB2	8	48.7±2.81 (8)	3.67±1.96 (8)	0.021±0.012 (8)	0.095±0.144 (8)	0.937±1.15 (7)	6.43±7.46 (7)	-0.21±2.9 (7)	-0.74±2.12 (7)
PB3	104	54±4.71 (102)	2.31±1.23 (102)	0.024±0.027 (102)	0.011±0.052 (104)	-0.141±1.42 (97)	7.05±12.8 (97)	-0.41±1.97 (68)	-0.65±1.61 (68)
PB3.5	161	55.3±3.61 (69)	2.1±0.923 (69)	0.037±0.110 (69)	0.005±0.037 (161)	0.052±1.82 (158)	11.3±55.1 (158)	-0.32±2.15 (50)	-0.82±1.72 (50)
PB3.8	160	55.4±3.64 (69)	2.05±0.947 (69)	0.026±0.025 (69)	0.005±0.037 (160)	0.051±1.84 (157)	11.3±55.3 (157)	-0.32±2.15 (50)	-0.82±1.72 (50)
PB4	196	54±4.84 (101)	2.33±1.45 (102)	0.020±0.013 (103)	0.006±0.038 (196)	0.078±1.82 (188)	11±51.3 (187)	-0.41±1.97 (68)	-0.65±1.61 (68)
PB4.1	160	55.1±3.7 (69)	2.06±0.924 (69)	0.020±0.009 (69)	0.005±0.037 (160)	-0.004±1.88 (158)	11.2±55.1 (158)	-0.32±2.15 (50)	-0.82±1.72 (50)
PB4.2	161	55.1±3.6 (69)	2.1±1.01 (69)	0.022±0.012 (69)	0.005±0.037 (161)	-0.025±1.87 (159)	11±54.9 (159)	-0.32±2.15 (50)	-0.82±1.72 (50)
PB4.5	162	55.3±3.73 (69)	2.13±0.912 (69)	0.020±0.009 (69)	0.005±0.037 (162)	-0.043±1.9 (160)	11.2±54.8 (160)	-0.32±2.15 (50)	-0.82±1.72 (50)
PB5	105	53.8±4.67 (102)	2.33±1.24 (103)	0.020±0.011 (103)	0.011±0.052 (105)	-0.302±1.6 (99)	5.69±6.57 (98)	-0.41±1.97 (68)	-0.65±1.61 (68)
O1	20	56.3±2.64 (20)	1.25±0.59 (20)	0.013±0.007 (20)	n/a	-0.426±1.01 (20)	5.35±4.3 (20)	n/a	n/a
O2	20	56.2±2.47 (20)	1.66±0.865 (20)	0.017±0.012 (20)	n/a	-0.356±0.948 (20)	5.45±4.31 (20)	n/a	n/a
O3	20	56.3±2.74 (20)	1.59±0.815 (20)	0.018±0.011 (20)	n/a	-0.356±0.948 (20)	5.45±4.31 (20)	n/a	n/a
O4	20	56.5±2.75 (20)	1.56±1.07 (20)	0.018±0.012 (20)	n/a	-0.304±0.951 (20)	5.5±4.35 (20)	n/a	n/a
O4.1	68	57.1±3.28 (68)	1.67±0.819 (68)	0.025±0.020 (68)	0.011±0.056 (68)	-0.181±1.12 (63)	5.98±4.34 (63)	-0.334±2.17 (49)	-0.79±1.72 (49)
O5	20	56.7±2.96 (20)	1.67±1.06 (20)	0.018±0.011 (20)	n/a	-0.251±0.826 (20)	5.05±4.2 (20)	n/a	n/a

6.4. Microbiological Results

This section contains the results for all microbiological assays including: *E. coli* and Ent counts in feces; TC, *E. coli* and Ent (FIB) counts in water samples broken out by sampling scheme; *Bacteroides* PCR results; bacterial pathogen assays; *E. coli* strain matching to the IEH fecal strain library; the use of TRFLP for fecal source ID; and results from testing the hand held PCR device.

6.4.1. Ent and *E. coli* in Fecal Samples

The samples collected for the fecal library (section 4.1) were tested for Ent and *E. coli* levels to determine ratios specific to certain fecal sources. In general the variation in counts was large, making any prediction of source based on Ent/*E. coli* ratio useless (Table 6.4.1-1).

Table 6.4.1-1. Counts of Ent and *E. coli* (MPN/g) in samples from the fecal library listed as means and geomeans ± standard deviations. N refers to the number of samples.

Source	FIB	N	Mean	Geomean
cat	Ent	2	$1.4 \pm 1.9 \times 10^6$	5.7 ± 1.0
	E. coli	4	$1.5 \pm 2.1 \times 10^7$	6.5 ± 1.4
cow	Ent	3	$3.2 \pm 2.0 \times 10^4$	4.4 ± 0.4
	E. coli	2	$6.4 \pm 9.0 \times 10^5$	4.5 ± 2.3
dog	Ent	8	$2.5 \pm 6.0 \times 10^6$	5.4 ± 1.0
	E. coli	9	$2.4 \pm 6.6 \times 10^6$	4.1 ± 2.1
duck	Ent	6	$1.4 \pm 3.1 \times 10^4$	2.8 ± 1.2
	E. coli	6	$8.4 \pm 11 \times 10^3$	3.5 ± 0.7
horse	Ent	14	$7.2 \pm 8.5 \times 10^6$	6.3 ± 1.0
	E. coli	12	$8.9 \pm 9.1 \times 10^6$	6.3 ± 1.2
pigeon	Ent	18	$6.1 \pm 14 \times 10^5$	4.0 ± 1.6
	E. coli	19	$2.7 \pm 7.6 \times 10^7$	4.7 ± 2.2
gull	Ent	9	$2.0 \pm 4.7 \times 10^6$	4.9 ± 1.4
	E. coli	11	$4.0 \pm 8.5 \times 10^6$	5.9 ± 0.8
sewage	Ent	3	$1.5 \pm 1.7 \times 10^5$	4.4 ± 1.6
	E. coli	3	$3.7 \pm 1.8 \times 10^6$	4.8 ± 2.0

6.4.2. Fecal Indicator Bacteria (FIB)

The comprehensive data set produced in this study included 2,043 records from 7/31/2007 through 5/25/2009. A record was defined as a unique visit to a sampling site with at least one FIB measurement. The tabulated summary statistics in Table 6.4.2-1 present the complete set of data collected between 7/31/2007 and 5/25/2009. Not all variables were observed for this entire data range. FIB counts are summarized below broken out by site and sampling scheme in later

tables with the number of samples for which data was collected (N), the mean, and the geomean with respective standard deviations. Graphs of AB411 exceedences are included where appropriate. Some samples may be included in more than one summary table.

Table 6.4.2-1. Summary statistics of FIB counts (MPN/100 mL) from all samplings broken out by sampling site. Standard deviations are listed in parentheses. N refers to the number of samples.

	TC			*E coli*			Ent		
Site	**N**	**Mean**	**Geomean**	**N**	**Mean**	**Geomean**	**N**	**Mean**	**Geomean**
PB1	8	141 (158)	1.91 (0.575)	8	24.1 (27.9)	2.3 (0.442)	8	7.53 (5.41)	0.812 (0.225)
PB2	7	361 (522)	2.05 (0.763)	7	31.9 (56)	1.16 (0.465)	8	14.6 (12.5)	1.04 (0.343)
PB3	103	250 (297)	2.14 (0.54)	103	113 (114)	1.13 (0.544)	102	18.2 (26.7)	1.04 (0.385)
PB3.5	161	412 (433)	2.4 (0.496)	161	234 (259)	1.79 (0.552)	160	51 (67.7)	1.38 (0.548)
PB3.8	160	792 (1220)	2.63 (0.495)	160	416 (713)	2.12 (0.536)	159	99.1 (245)	1.53 (0.625)
PB4	196	1220 (2440)	2.69 (0.6)	196	565 (989)	2.33 (0.525)	195	104 (185)	1.53 (0.651)
PB4.1	159	834 (2110)	2.48 (0.624)	159	387 (676)	2.39 (0.583)	158	79.6 (160)	1.48 (0.601)
PB4.2	161	455 (899)	2.29 (0.591)	161	280 (699)	2.16 (0.667)	160	84 (357)	1.34 (0.603)
PB4.5	162	247 (294)	2.09 (0.582)	162	128 (149)	2.01 (0.644)	160	57.1 (166)	1.29 (0.572)
PB5	104	267 (809)	1.94 (0.628)	104	127 (455)	1.77 (0.608)	103	27.1 (68.6)	1.07 (0.455)
C1	8	15800 (9380)	4.08 (0.399)	8	646 (597)	1.64 (0.578)	8	2290 (2540)	3.06 (0.576)
C2	8	8870 (8920)	3.67 (0.602)	8	487 (780)	1.86 (0.663)	8	825 (1460)	2.43 (0.671)
C3	8	14500 (11000)	3.92 (0.586)	8	1150 (1630)	2.67 (0.359)	8	2420 (3890)	2.76 (0.929)
L1	46	12500 (8390)	3.94 (0.448)	48	356 (501)	2.25 (0.647)	48	1120 (1840)	2.25 (0.975)
O1	20	370 (1530)	1.01 (0.799)	20	5.5 (1.54)	2.38 (1.02)	19	5 (0)	0.699 (0)
O2	20	75.6 (232)	1.25 (0.591)	20	12 (16.1)	0.729 (0.093)	19	6.61 (5.92)	0.756 (0.19)
O3	20	47.7 (69.5)	1.31 (0.574)	20	25.2 (47.9)	0.903 (0.337)	19	9.87 (11.8)	0.857 (0.292)
O4	20	1260 (5400)	1.42 (0.944)	20	232 (975)	1.07 (0.475)	19	6.06 (3.61)	0.747 (0.152)
O4.1	68	819 (2510)	2.35 (0.688)	68	184 (303)	1.1 (0.712)	67	27 (83.8)	1.05 (0.433)
O5	20	85 (239)	1.31 (0.629)	20	17.4 (25.4)	0.982 (0.419)	19	7.13 (5.98)	0.788 (0.203)

Data from the daily sampling effort in summer 2008 showed the average counts of all FIB were highest next to the pier (Table 6.4.2-2). A graphical analysis showing AB411 exceedences in relation to daily high tides also indicated a relationship between the tide cycle and high FIB counts (Figure 6.4.2-1).

Table 6.4.2-2. Summary statistics of FIB counts from the 2008 daily summer samplings broken out by sampling site. Standard deviations are listed in parentheses. N refers to the number of samples.

	TC			*E coli*			Ent		
Site	**N**	**Mean**	**Geomean**	**N**	**Mean**	**Geomean**	**N**	**Mean**	**Geomean**
PB3	61	294 (272)	2.26 (0.508)	61	146 (127)	1.97 (0.48)	60	15.7 (16.1)	1.04 (0.346)
PB3.5	61	449 (504)	2.43 (0.517)	61	211 (184)	2.13 (0.488)	60	26.7 (29.3)	1.19 (0.456)
PB3.8	61	706 (794)	2.6 (0.514)	61	342 (461)	2.29 (0.499)	60	52.2 (103)	1.28 (0.565)
PB4	61	868 (1070)	2.71 (0.462)	61	414 (608)	2.38 (0.449)	60	48.1 (68.7)	1.41 (0.485)
PB4.1	60	1120 (3250)	2.46 (0.687)	60	465 (935)	2.13 (0.713)	59	41.7 (73.8)	1.25 (0.533)
PB4.2	61	407 (443)	2.32 (0.57)	61	240 (264)	2.06 (0.602)	60	24.1 (27.3)	1.15 (0.445)
PB4.5	61	288 (373)	2.15 (0.57)	61	134 (158)	1.82 (0.583)	59	25.6 (34.9)	1.17 (0.443)
PB5	61	250 (589)	2.06 (0.537)	61	179 (588)	1.78 (0.583)	60	19.1 (24.1)	1.07 (0.396)
O4.1	60	920 (2660)	2.43 (0.679)	60	204 (318)	1.93 (0.658)	59	29.5 (89.1)	1.08 (0.443)

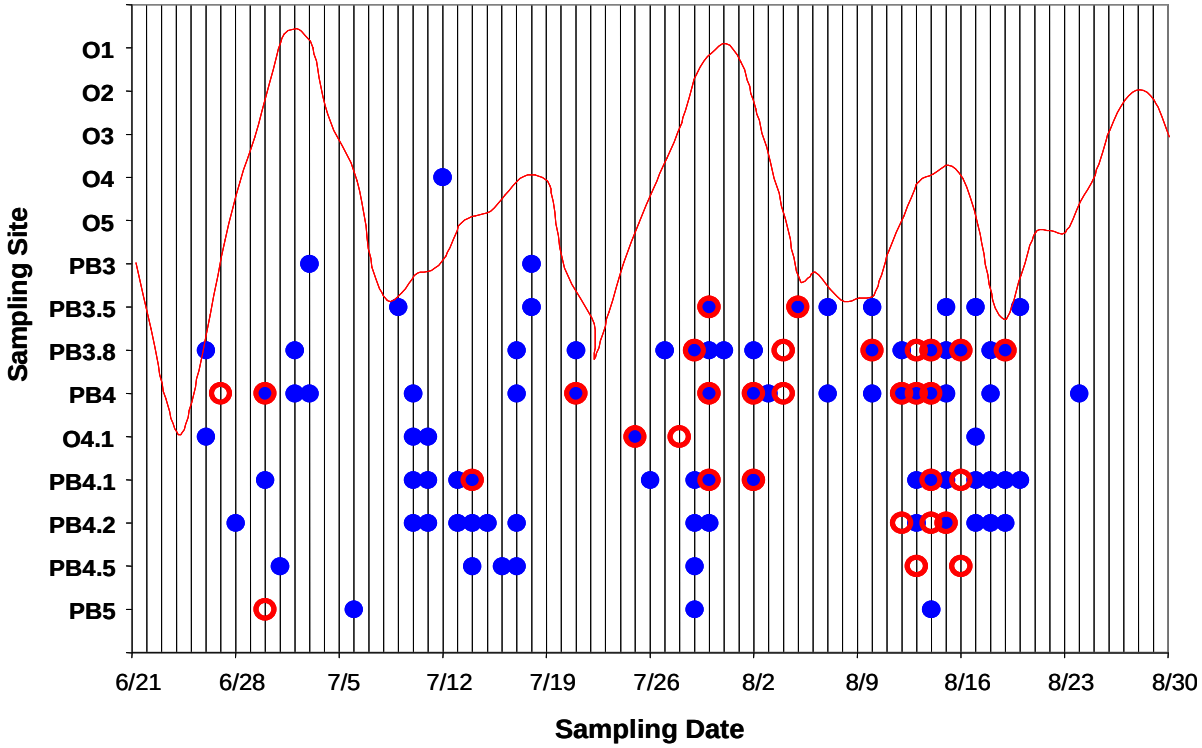


Figure 6.4.2-1. FIB counts exceeding AB411 limits from the 2008 summer samplings broken out by sampling site. The filled blue circles indicate *E. coli* exceedences and open red circles indicate Ent exceedences. The red line represents the relative highest watermark for each day during the sampling period.

Data from the hourly sampling efforts in summer 2008 again showed the average counts of all FIB were highest next to the pier (Table 6.4.2-3). Again, a graphical analysis showing AB411 exceedences in relation to tide levels also indicated a relationship between the tide cycle and high FIB counts although this relationship was less notable in the July 30th to August 1st hourly sampling run (Figure 6.4.2-2).

Table 6.4.2-3. Summary statistics of FIB counts from the 2008 hourly samplings broken out by sampling site. Standard deviations are listed in parentheses. N refers to the number of samples.

	TC			*E coli*			Ent		
Site	**N**	**Mean**	**Geomean**	**N**	**Mean**	**Geomean**	**N**	**Mean**	**Geomean**
PB3.5	98	424 (410)	2.42 (0.484)	98	270 (306)	2.17 (0.532)	98	68.1 (79.6)	1.52 (0.562)
PB3.8	97	902 (1450)	2.69 (0.488)	97	485 (832)	2.42 (0.484)	97	130 (299)	1.69 (0.616)
PB4	97	1770 (3240)	2.88 (0.56)	97	821 (1280)	2.59 (0.545)	97	163 (235)	1.74 (0.699)
PB4.1	97	708 (1040)	2.5 (0.591)	97	386 (665)	2.2 (0.649)	97	101 (195)	1.6 (0.605)
PB4.2	98	488 (1100)	2.3 (0.582)	98	322 (873)	2.04 (0.647)	98	112 (448)	1.42 (0.642)
PB4.5	98	219 (225)	2.06 (0.577)	98	133 (153)	1.78 (0.628)	98	53.1 (85.2)	1.32 (0.584)

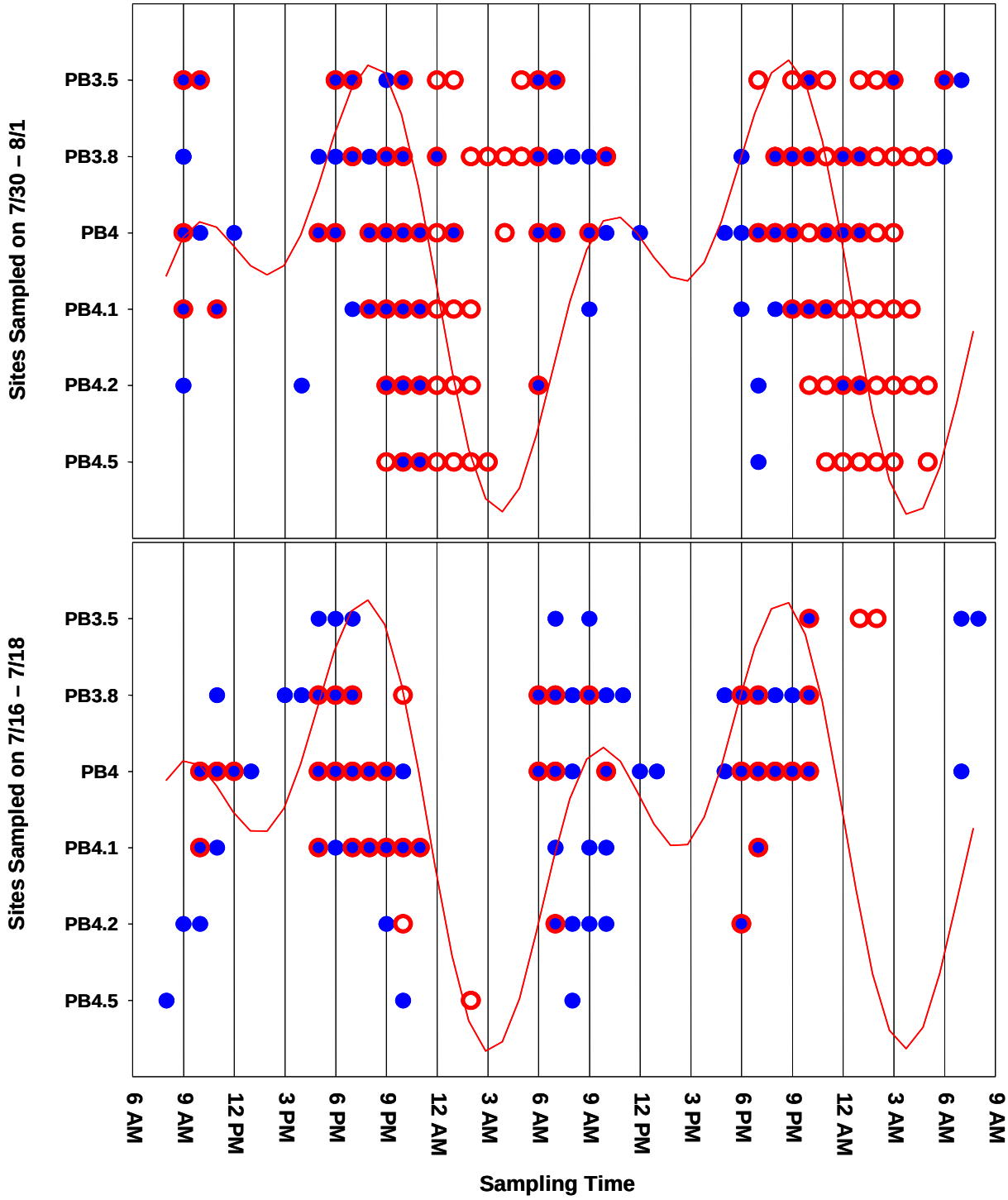


Figure 6.4.2-2. FIB counts exceeding AB411 limits from the two hourly samplings in 2008, broken out by sampling site. The filled blue circles indicate *E. coli* exceedences and open red circles indicate Ent exceedences. The red line represents the relative tide level for each hour during the sampling period.

Data from the weekly sampling effort from 5/6/2008 to 5/25/2009 showed the average counts of all FIB were highest from the lagoon site (L1), followed by PB4 next to the pier (Table 6.4.2-4). A graphical analysis showing AB411 exceedences showed the weekly measurements only captured *E. coli* exceedences at PB4 and L1 (Figure 6.4.2-3).

Table 6.4.2-4. Summary statistics of FIB counts from the 5/6/2008 to 5/25/2009 weekly samplings broken out by sampling site. Standard deviations are listed in parentheses. N refers to the number of samples.

	TC			*E coli*			Ent		
Site	**N**	**Mean**	**Geomean**	**N**	**Mean**	**Geomean**	**N**	**Mean**	**Geomean**
L1	46	12500 (8390)	3.94 (0.45)	48	356 (501)	2.30 (0.44)	48	1120 (1840)	2.25 (0.98)
PB3	52	211 (299)	2.06 (0.53)	52	91 (86)	1.68 (0.58)	51	21 (34)	1.06 (0.42)
PB4	50	540 (854)	2.31 (0.65)	50	255 (381)	2.02 (0.63)	49	43 (96)	1.21 (0.53)
PB5	52	266 (959)	1.75 (0.71)	52	58 (70)	1.46 (0.54)	51	34 (94)	1.06 (0.50)

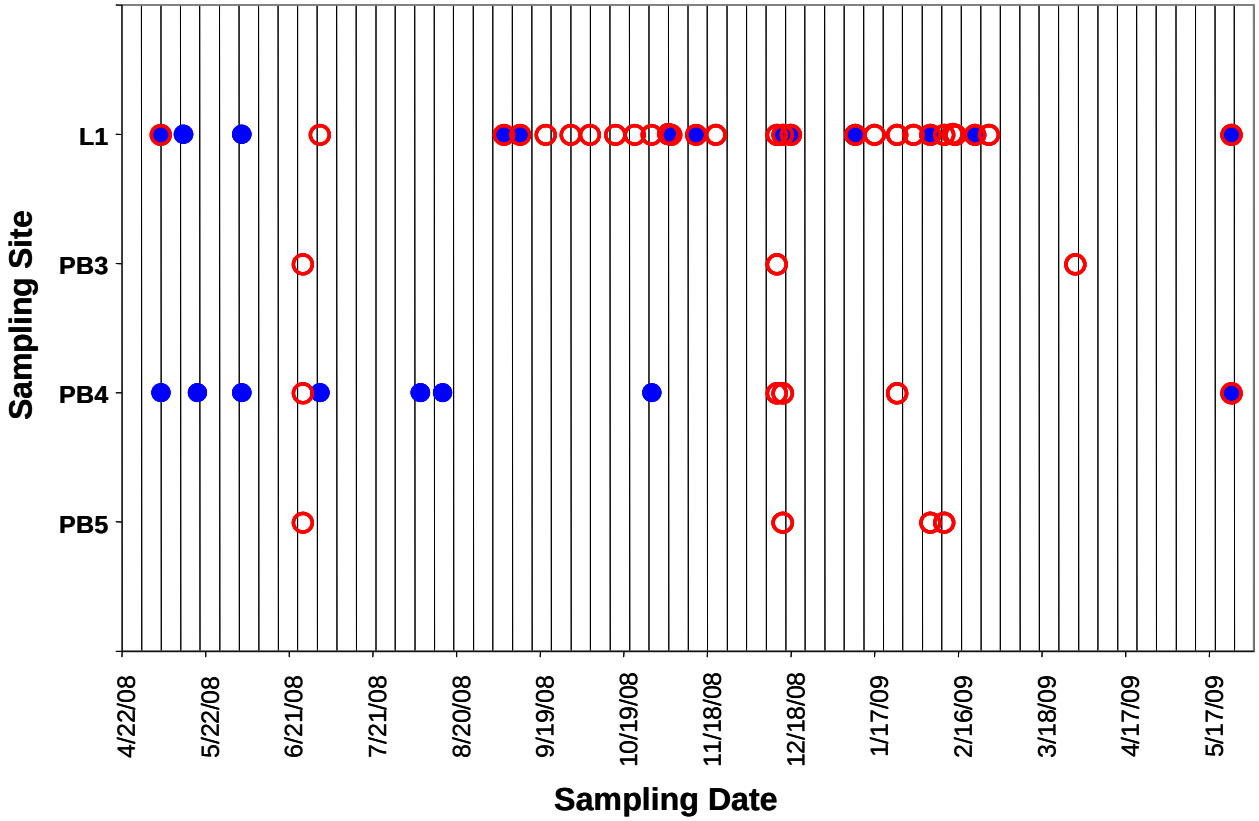


Figure 6.4.2-3. FIB counts exceeding AB411 limits from the 2008 weekly samplings broken out by sampling site. The filled blue circles indicate *E. coli* exceedences and open red circles indicate Ent exceedences.

Data from the 2009 rain event samplings showed the average counts of all FIB were highest in the creek and lagoon, followed by sites to the north of the pier, from PB4.2 to PB5 (Table 6.4.2-5). This is an unexpected result that may indicate a contribution from street runoff at these sites.

Table 6.4.2-5. Summary statistics of FIB counts from the 2009 rain event samplings broken out by sampling site. Standard deviations are listed in parentheses. N refers to the number of samples.

	TC			*E coli*			Ent	
Site	**N**	**Mean**	**Geomean**	**N**	**Mean**	**Geomean**	**N**	**Mean**	**Geomean**
C1	8	15800 (9380)	4.08 (0.399)	8	646 (597)	2.67 (0.359)	8	2290 (2540)	3.06 (0.576)
C2	8	8870 (8920)	3.67 (0.602)	8	487 (780)	2.25 (0.647)	8	825 (1460)	2.43 (0.671)
C3	8	14500 (11000)	3.92 (0.586)	8	1150 (1630)	2.38 (1.02)	8	2420 (3890)	2.76 (0.929)
L1	7	20000 (7270)	4.26 (0.208)	8	740 (620)	2.71 (0.419)	8	2990 (2720)	3.23 (0.539)
PB1	8	141 (158)	1.91 (0.575)	8	24.1 (27.9)	1.16 (0.465)	8	7.53 (5.41)	0.812 (0.225)
PB2	7	361 (522)	2.05 (0.763)	7	31.9 (56)	1.13 (0.544)	8	14.6 (12.5)	1.04 (0.343)
PB3	8	113 (118)	1.86 (0.446)	8	21.5 (31.1)	1.09 (0.443)	8	13.8 (7.05)	1.08 (0.269)
PB3.5	8	117 (93.6)	1.96 (0.343)	8	69.4 (110)	1.47 (0.594)	8	28 (32.8)	1.17 (0.518)
PB3.8	8	238 (211)	2.22 (0.426)	8	61.2 (69.1)	1.53 (0.516)	8	38.1 (32.6)	1.38 (0.507)
PB4	8	207 (175)	2.12 (0.484)	8	83.7 (67.2)	1.72 (0.513)	8	73.9 (156)	1.32 (0.65)
PB4.1	8	489 (541)	2.35 (0.681)	8	248 (382)	1.95 (0.747)	8	100 (111)	1.67 (0.646)
PB4.2	8	418 (467)	2.21 (0.796)	8	117 (158)	1.58 (0.771)	8	144 (247)	1.6 (0.755)
PB4.5	8	380 (367)	2.33 (0.557)	8	95.1 (112)	1.61 (0.642)	8	317 (659)	1.83 (0.811)
PB5	8	994 (2380)	2.05 (0.956)	8	74.9 (84)	1.51 (0.673)	8	152 (207)	1.75 (0.759)
O4.1	8	65 (36.3)	1.71 (0.367)	8	35 (26.4)	1.36 (0.485)	8	8.82 (8.97)	0.835 (0.283)

6.4.3. *Bacteroides* Fecal Source Marker PCR

Bacteroides source marker PCR results are summarized below broken out by site in tables with the number of samples for which data was collected (N), the number of positive and negative results and a percentage of positive results. Graphs of positive results are included where appropriate.

6.4.3.1. Human and Dog *Bacteroides*

By far the most samples were found positive for human and dog *Bacteroides* as compared to the other markers tested. When results were broken out by site, human *Bacteroides* were detected in 0 % to 43 % of samples from a single site (Table 6.4.3.1-1) with an average of 11 % positive samples across all sites. Similarly, dog *Bacteroides* were detected in 0 % to 86 % of samples from a single site (Table 6.4.3.1-1) with an average of 14 % positive samples across all sites. The C1 site (Cypress St. Bridge) had the highest % positive for both human and dog *Bacteroides*, perhaps because samples were only taken at C1 during rain events. No samples contained enough *Bacteroides* (human or dog) to create the equivalent of an AB411 exceedence in the corresponding FIB count.

Table 6.4.3.1-1. Summary of human and dog *Bacteroides* PCR results broken out by sampling site. N refers to the number of samples.

	Human *Bacteroides*				Dog *Bacteroides*			
Site	**N**	**Negative**	**Positive**	**% Positive**	**N**	**Negative**	**Positive**	**% Positive**
L1	48	43	5	10 %	48	38	10	21 %
PB1	8	8	0	0 %	8	7	1	13 %
PB2	8	7	1	13 %	8	8	0	0 %
PB3	103	93	10	10 %	103	91	12	12 %
PB3.5	69	62	7	10 %	69	58	11	16 %
PB3.8	69	60	9	13 %	69	61	8	12 %
PB4	105	100	5	5 %	105	93	12	11 %
PB4.1	66	59	7	11 %	66	60	6	9 %
PB4.2	69	62	7	10 %	69	65	4	6 %
PB4.5	70	60	10	14 %	70	66	4	6 %
PB5	104	97	7	7 %	104	92	12	12 %
O1	20	19	1	5 %	20	17	3	15 %
O2	20	15	5	25 %	20	18	2	10 %
O3	20	19	1	5 %	20	17	3	15 %
O4	20	17	3	15 %	20	16	4	20 %
O4.1	68	61	7	10 %	68	64	4	6 %
O5	20	18	2	10 %	20	18	2	10 %
C1	7	4	3	43 %	7	1	6	86 %
C2	7	7	0	0 %	7	7	0	0 %
C3	8	7	1	13 %	8	8	0	0 %

By breaking out the results for Human and Dog *Bacteroides* by site and date for the summer daily sampling scheme it can be seen that very few samples (0.5 % of all positive samples) were positive for both *Bacteroides* markers (Figure 6.4.3.1-1). Interestingly, there seems to be some periodicity to the occurrence of positive samples and large areas of the beach often showed up with positive samples on the same days. Of particular note, a large number of samples were positive for Human *Bacteroides* during the days surrounding the July Fourth holiday (Figure 6.4.3.1-1).

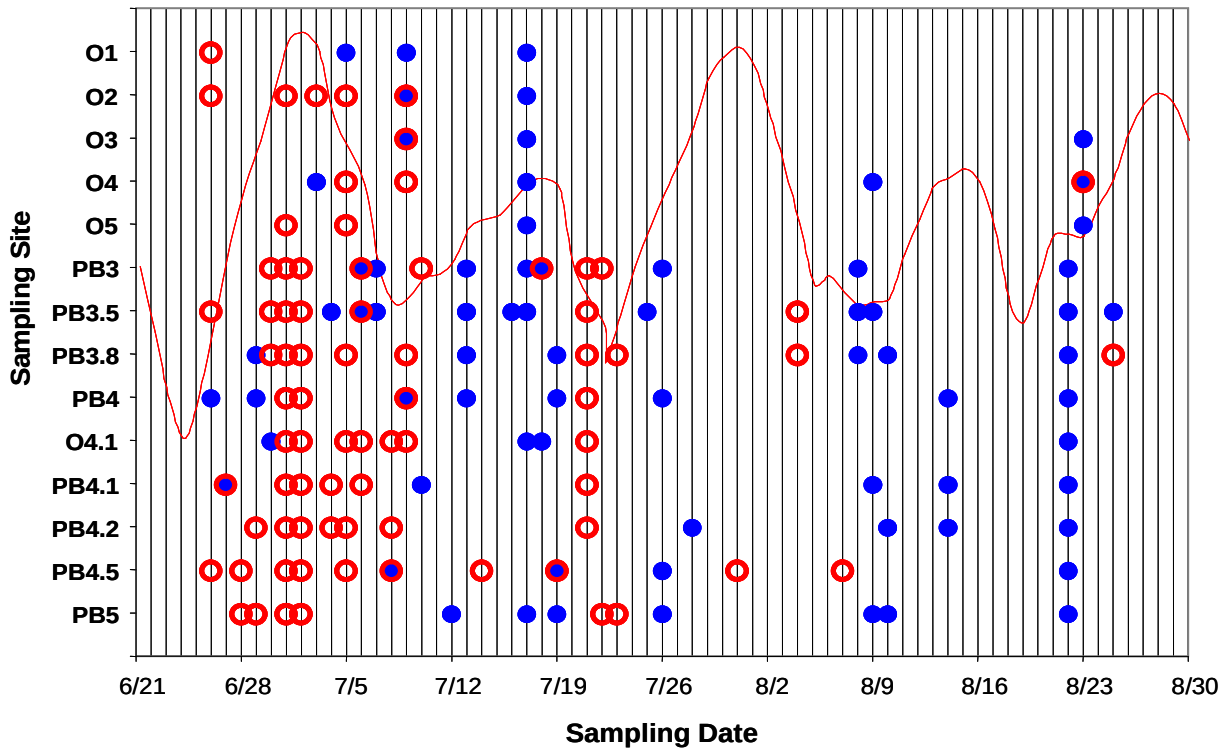


Figure 6.4.3.1-1. Dog and Human *Bacteroides* PCR results from the 2008 summer samplings broken out by sampling site. Filled blue circles indicate positive samples for Dog *Bacteroides*, open red circles indicate positive samples for Human *Bacteroides*. The red line represents the relative highest watermark for each day during the sampling period.

6.4.3.2. Cow and Horse *Bacteroides*

None of the 909 samples evaluated for horse-specific *Bacteroides* were identified as being positive for this marker. This could be a result of the low detection limit observed for horse feces using this method (Table 5.2.2-1). Of the 270 samples (rain events and weekly year-round samples only) tested with the cow-specific primers for *Bacteroides*, those taken during the rain events at the creek/lagoon sites were often positive, from 79 % at L1 to 100 % at two of the upstream creek sites (Table 6.4.3.2-1). Conversely, most of the samples from beach sites were negative for cow *Bacteroides*.

Table 6.4.3.2-1. Summary of Cow *Bacteroides* PCR results broken out by sampling site. N refers to the number of samples.

Site	N	Negative	Positive	% Positive
L1	47	10	37	79%
PB1	8	8	0	0%
PB2	8	8	0	0%
PB3	46	45	1	2%
PB3.5	8	7	1	13%
PB3.8	8	8	0	0%
PB4	48	47	1	2%
PB4.1	6	6	0	0%
PB4.2	7	5	2	29%
PB4.5	8	8	0	0%
PB5	46	45	1	2%
O4.1	8	7	1	13%
C1	7	1	6	86%
C2	7	0	7	100%
C3	8	0	8	100%
Total	270	205	65	24%

6.4.4. Bacterial Pathogens

Nine waterborne pathogens were chosen for this study. They were categorized into four groups: 1) waterborne protozoan pathogens, represented by *Cryptosporidium* and *Giardia* (in section 6.4.5); 2) pathogens associated with wound infection, represented by *Vibrio vulnificus*, *V. parahaemolyticus*, *Aeromonas* spp. and *Pseudomonas* spp.; 3) pathogens associated with birds, represented by *Salmonella* spp. and *Campylobacter* spp.; and 4) pathogens associated with humans, represented by *Shigella* spp. Selection of pathogens for this pilot study was based on the following criteria: 1) a significant number of strains within the species have the ability to cause disease, 2) a common route of infection is through exposure and/or consumption of water, and 3) the reservoirs are fairly specific to certain hosts or habitats, therefore their presence may provide a hint as to the source of pollution.

Twenty-four samplings were carried out during spring tides to determine the prevalence of pathogens at PB4 and L1 sites. In addition, a total of eight samplings were also carried out at the beginning and end of four separate rain events. Preliminary data analyses were carried out to determine the presence/absence of bacterial pathogens at these sites. All bacterial pathogens were detected more often at L1 than at PB4 (Table 6.4.4-1). During spring tide, exclude rain events, almost all (92%) L1 samples contained *Aeromonas* and *Shigella* spp. The majority also contained *Campylobacter* spp. (75%), *Pseudomonas* spp. (70%), *Salmonella* spp. (83%), *Shigella* spp. (83%), *Vibrio parahaemolyticus* (79%) and *V. vulnificus* (75%). On the other

hand, approximately half (40-60%) of the PB4 samples contained *Aeromonas* spp., *Pseudomonas* spp., *Salmonella* spp., *Campylobacter* spp., *V. parahaemolyticus* and *V. vulnificus*. The incidence of *P. aeruginosa* in PB4 was very low.

During or after the rain events, *Aeromonas* spp., *Pseudomonas* spp., *P. aeruginosa,* and *V. vulnificus* were found more often at PB4 compared to their presence during non-rain event spring tides. Among these four pathogens, the last three also showed increased prevalence in L1 during rain events as did *Salmonella* and *Campylobacter* spp. Collectively, L1 samples obtained during rain events had the highest incidence of bacterial pathogens. This is not unexpected as some of these pathogens can be found in human and animal feces likely brought to L1 via the watershed.

Interestingly, the occurrence of *V. parahaemolyticus* and *Shigella* spp did not increase during rain events. Since *V. parahaemolyticus* is a natural inhabitant in brackish water worldwide and is rarely found in agricultural runoff, it is possible that the increase in runoff not only failed to produce higher levels of this pathogen, but instead may have produced a diluting effect. In contrast, *Shigella* is commonly found in human feces so its presence may be expected to increase during rain events, though it did not.

Table 6.4.4-1. Presence of bacterial pathogens at PB4 and L1 during spring tides and rain event samplings.

	Presence in spring tide samples (N)		Presence in rain event samples (N)		Presence in all samples (N)	
Pathogen	**PB4**	**L1**	**PB4**	**L1**	**PB4**	**L1**
Aeromonas spp.	58 % (24)	92 % (24)	100 % (8)	88 % (8)	69 % (32)	91 % (32)
Pseudomonas spp.	41 % (22)	70 % (23)	71 % (7)	100 % (7)	48 % (29)	77 % (30)
P. aeruginosa	4 % (23)	50 % (24)	38 % (8)	75 % (8)	13 % (31)	56 % (32)
Salmonella spp.	61 % (23)	83 % (23)	50 % (8)	100 % (8)	58 % (31)	87 % (31)
Campylobacter spp.	52 % (23)	75 % (24)	25 % (8)	88 % (8)	45 % (31)	78 % (32)
V. parahaemolyticus	42 % (24)	79 % (24)	25 % (8)	63 % (8)	38 % (32)	75 % (32)
V. vulnificus	57 % (23)	75 % (24)	63 % (8)	100 % (8)	58 % (31)	81 % (32)
Shigella spp.	83 % (23)	92 % (24)	75 % (8)	88 % (8)	81 % (31)	91 % (32)

The concentration of bacterial pathogens in these same samples was also determined, through the use of separate assays in the case of some pathogens (Table 6.4.4-2). When multiple quantitative assays were employed, weight was given to the more reliable methods when major discrepancies occurred between methods. Either CFU/100 mL or MPN/100 mL is presented for each bacterial

pathogen, depending on the assay used. Cell counts based on membrane filtration method (CFU results) were adjusted after taking calculated filtration efficiencies into account.

Table 6.4.4-2. Summary statistics of bacterial pathogens inclusive of spring tides and rain events samplings. Results are presented as: CFU/100 mL or MPN/100 mL; Mean ± SD (N)

	PB4			L1		
Pathogen	**N**	**Mean**	**Geomean**	**N**	**Mean**	**Geomean**
Aeromonas spp.	32	104 ± 359	0.65 ± 1.31	32	2997 ± 3091	2.87 ± 1.37
Campylobacter spp.	31	2 ± 3	-0.31 ± 0.80	32	177 ± 412	1.07 ± 1.33
Pseudomonas spp.	30	986 ± 1670	2.51 ± 0.69	31	66593 ± 128474	4.32 ± 0.69
P. aeruginosa	31	0 ± 1	-0.83 ± 0.44	32	9 ± 21	0.04 ± 1.01
Salmonella spp.	31	20 ± 43	0.84 ± 0.70	32	499 ± 550	2.39 ± 0.59
Shigella spp.	31	81 ± 300	0.52 ± 1.03	32	213 ± 489	1.20 ± 1.09
V. parahaemolyticus	32	15 ± 44	0.11 ± 0.91	32	1466 ± 3797	1.87 ± 1.57
V. vulnificus	31	72 ± 306	0.38 ± 1.09	32	15994 ± 44057	2.67 ± 1.84

As might be expected, the lagoon (L1) consistently harbored significantly higher concentrations of all pathogens (all T-test p-values were < 0.015) compared to the ocean next to the pier (PB4).

To determine if any pathogens were appearing in concert (indicating a common origin) all pairwise correlations between pairs of pathogens were computed for each site (Table 6.4.4-3). Based on a sample size of 32, correlations with magnitude larger than 0.337 were significant at the 0.05 level using Pearson's z-test for correlation. Because of the large number of pairs, the Bonferroni adjusted level 0.05 tests are significant for correlations greater than 0.495 in magnitude. After Bonferroni correction, only two pairs of pathogens showed significantly correlated abundances. *Pseudomonas* spp. and *Aeromonas* spp. abundances were correlated at the PB4 site though issues with obtaining accurate counts for *Pseudomonas* spp. may play a role in this (see section 6.4.4.3.). Counts for *P. aeruginosa* and *Salmonella* spp. were correlated at the L1 site, possibly indicating a common source for these pathogens.

Table 6.4.4-3. Coefficients from a pairwise comparison testing of pathogen correlations at each site. Significant results are in bold, based on a Bonferroni adjusted significance level. The top right triangle contains data for L1 while the bottom left triangle contains data for PB4

Pathogen	*Aeromonas* spp.	*Campylobacter* spp.	*Pseudomonas* spp	*P. aeruginosa*	*Salmonella* spp.	*Shigella* spp.	*V. parahaemolyticus*	*V. vulnificus*
Aeromonas spp.		–0.097	0.190	0.004	0.115	–0.141	–0.047	–0.398
Campylobacter spp.	0.087		–0.185	0.309	0.025	–0.008	–0.097	0.346
Pseudomonas spp.	**0.524**	0.149		0.177	0.396	–0.207	0.202	–0.211
P. aeruginosa	0.306	0.077	0.336		**0.585**	–0.307	–0.005	0.233
Salmonella spp.	0.213	0.139	0.265	0.067		–0.313	–0.046	0.022
Shigella spp.	–0.276	0.012	–0.285	–0.009	–0.226		0.447	–0.052
V. parahaemolyticus	0.052	–0.029	–0.008	–0.140	–0.238	0.027		–0.166
V. vulnificus	0.233	–0.089	0.174	–0.079	0.282	0.022	0.279	

6.4.4.1. *Aeromonas* spp.

Throughout the sampling year, with the exception of a few non-detect samples, *Aeromonas* spp. was consistently present at L1 and exceeded the level found at PB4 (Figure 6.4.4.1-1). Although *Aeromonas* was found in 85% of the pigeon feces tested (Table 6.4.4.7-1) its occurrence at PB4 was somewhat sporadic. The infective dose of *Aeromonas* via ingestion of contaminated water or food is unknown. Though scuba divers appeared to get an infection after ingesting low levels of *A. hydrophila*, voluntary studies suggested that the infective dose is high (FDA BBB). If the true oral infective dose of *Aeromonas* spp is indeed high, a large amount of seawater near PB4 (hundreds of liters) must be ingested to acquire the infection, which is unlikely to happen for most visitors to Pismo Beach. Nevertheless, the infective dose for wound infection is presumably very low. Open wound exposure to seawater may present a risk.

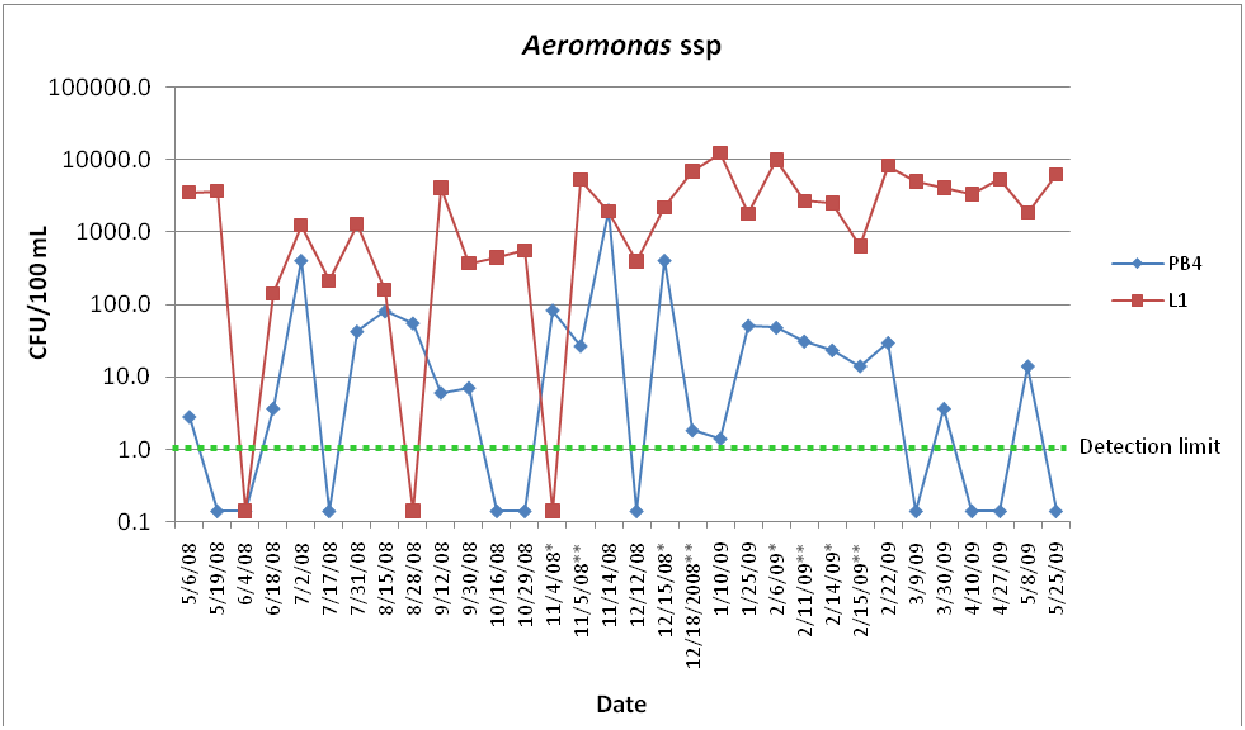


Figure 6.4.4.1-1. Cell count of *Aeromonas* spp. at PB4 and L1 from May 2008 to May 2009. All samples were collected during spring tides, during rain events (*) or immediately after rain events (**). Samples with undetectable level of the pathogen were arbitrarily given a value close to zero and are shown below the detection limit (dotted line).

6.4.4.2. *Campylobacter* spp.

Overall, the occurrence of *Campylobacter* spp was low at PB4; all PB4 samples positive for *Campylobacter* had concentrations of ≤10 MPN/100 mL (Figure 6.4.4.2-1). Once again, pigeon feces were found to occasionally harbor this pathogen (Table 6.4.4.7-1) but other birds present in the area were not tested. Much higher concentrations of *Campylobacter* spp. were seen at L1, where many ducks, seagulls and other birds were frequently seen. Ingestion of as little as 500 cells of *Campylobacter* spp. has been implicated in foodborne outbreaks of campylobacteriosis (FDA BBB). Given the low concentration (~2 MPN/100 mL) at PB4, it seems unlikely that visitors to Pismo Beach would acquire campylobacteriosis, unless a few liters of seawater were ingested. *Campylobacter* spp is not a major cause of wound infection.

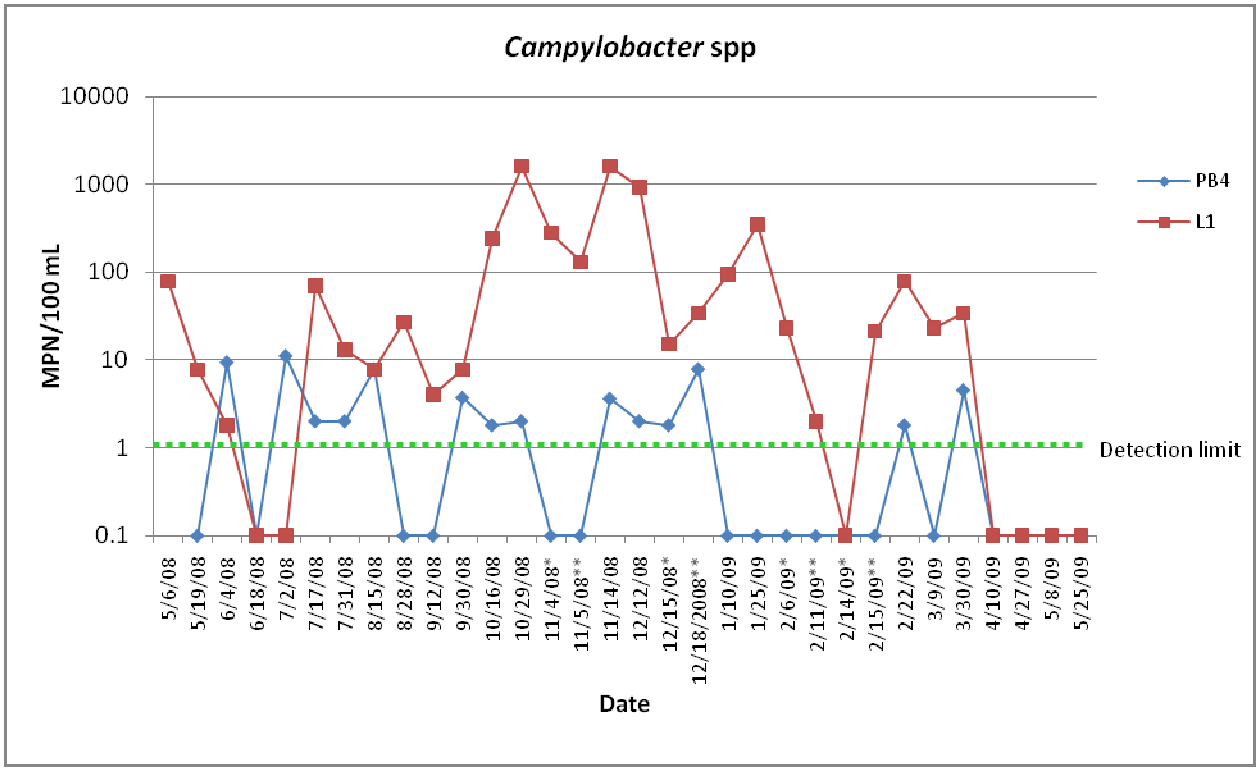


Figure 6.4.4.2-1. Cell count of *Campylobacter* spp in PB4 and L1 from May 2008 to May 2009. All samples were collected during spring tides, during rain events (*) or immediately after rain events (**). Samples with undetectable levels of the pathogen were arbitrarily given a value close to zero and are shown below the detection limit (dotted line).

6.4.4.3. *Pseudomonas* spp. and *P. aeruginosa*

Membrane filtration followed by filter placement on King's agar with Irgasan (an antibacterial chemical) was used to estimate *Pseudomonas* spp. concentrations. However, this medium is not totally selective for *Pseudomonas* spp as some species have natural resistance to Irgasan such as those within the *Serratia* and *Yersinia* genera (Flint and Hartley, 1996; Fukushima and Gomyoda, 1986). Therefore, CFU/100 mL results of *Pseudomonas* spp. shown here may be an overestimate to some degree (Figure 6.4.4.3-1). An immunoassay following selective enrichment was used to confirm presence/absence data for *Pseudomonas* spp. According to the presence/absence assay, *Pseudomonas* spp. occurred at PB4 and L1 in 48% and 77% of the samples, respectively (Table 6.4.4-1). The counts of *Pseudomonas* spp. at PB4 and L1 are consistent with the difference between sites but are clearly detecting more than just *Pseudomonas* spp. (Figure 6.4.4.3-1).

Both membrane filtration and MPN were used to quantify *P. aeruginosa*:. Species specific PCR (Tyler et al., 1995) was performed on 38 isolates representing 12 sampling batches obtained from the membrane filtration method, and 36 (95%) isolates or 11 (92%) sampling batches were confirmed as *P. aeruginosa*. Despite this high accuracy, the MPN method was selected for further analyses as it yielded more conservative results. Overall, the occurrence of *P. aeruginosa* appeared higher in the winter. *P. aeruginosa* is capable of causing wound infection and is a

primary causative agent of ear infection (otitis externa) in recreational waters. A study with whirlpools suggested that the infective dose is >1,000 organisms (Price and Ahearn, 1988), though the authors also realized a cell count of *P. aeruginosa* of <1 cell / mL could constitute a health hazard. In another previous study, swimmers acquired otitis externa when exposed to fresh water lakes containing as low as 2 CFU/100 mL (van Asperen et al., 1995). Based on our MPN and CFU estimates, it is quite possible to acquire *P. aeruginosa* infection from both PB4 and L1.

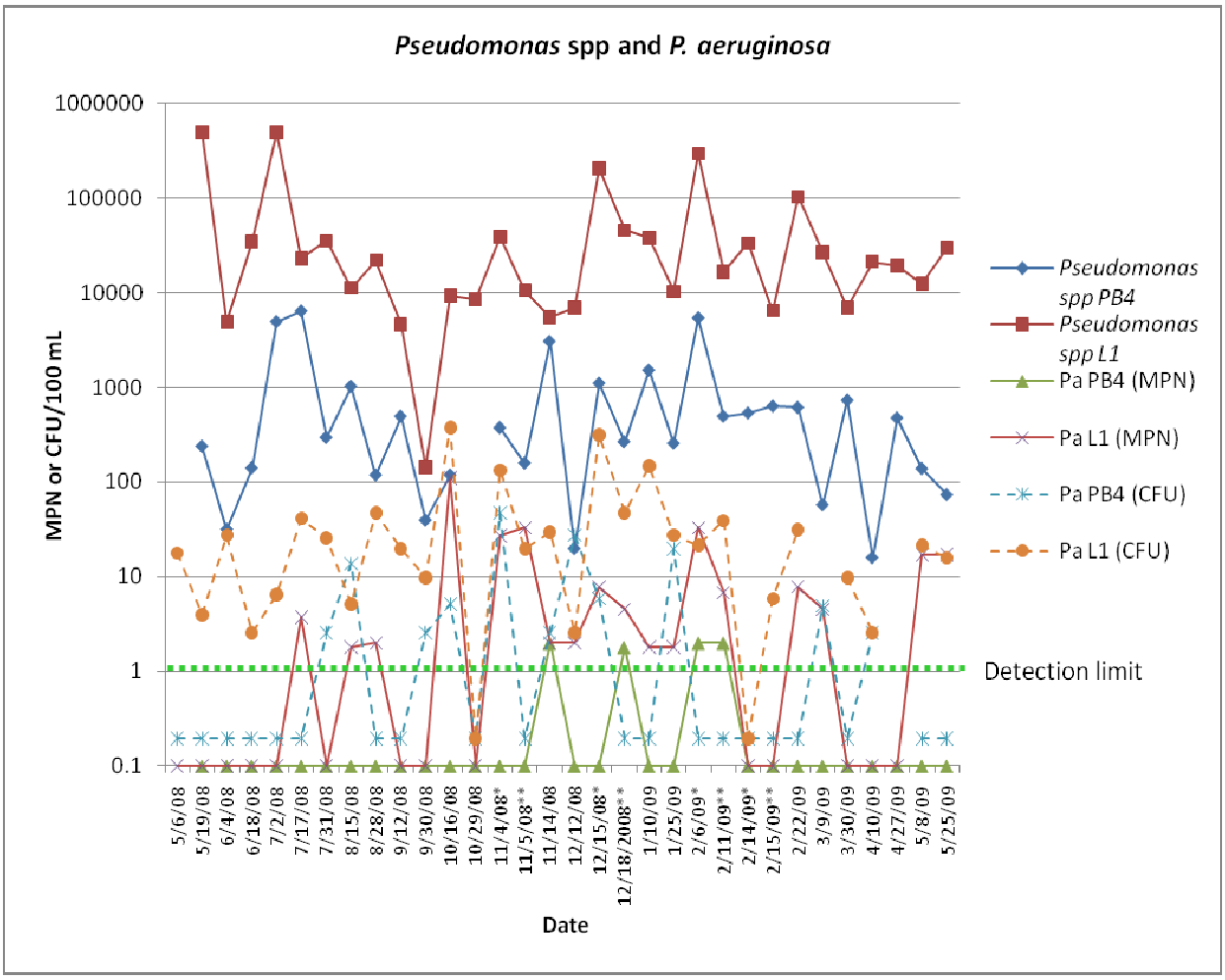


Figure 6.4.4.3-1. Cell count of *Pseudomonas* spp. (CFU/100 mL) and *P. aeruginosa* (MPN and CFU/100 mL) at PB4 and L1 from 5/6/2008 to 5/25/2009. All samples were collected during spring tides, during rain events (*) or immediately after rain events (**). Samples with undetectable levels of the pathogen were arbitrarily given a value close to zero and are shown below the detection limit (dotted line).

6.4.4.4. *Salmonella* spp.

The presence/absence of *Salmonella* spp. in samples was determined by an immunoassay (LATEX) following enrichment. This was expected to provide the most accurate measurement of the prevalence of *Salmonella*. However, we were later informed by the supplier that the

LATEX kit batches we used during the sampling year were defective. Therefore, we used another qualitative method (2 selective enrichment steps followed by streaking on a selective agar plate) and a quantitative method (MPN using a selective broth followed by streaking on a selective agar plate for confirmation). In a further complication, only 1 out of 18 isolates representing 12 sampling batches obtained from the selective agar was confirmed as *Salmonella* via genus specific PCR (Kwang et al., 1996). This suggests the concentrations shown in Figure 6.4.4.4-1 may be overestimates.

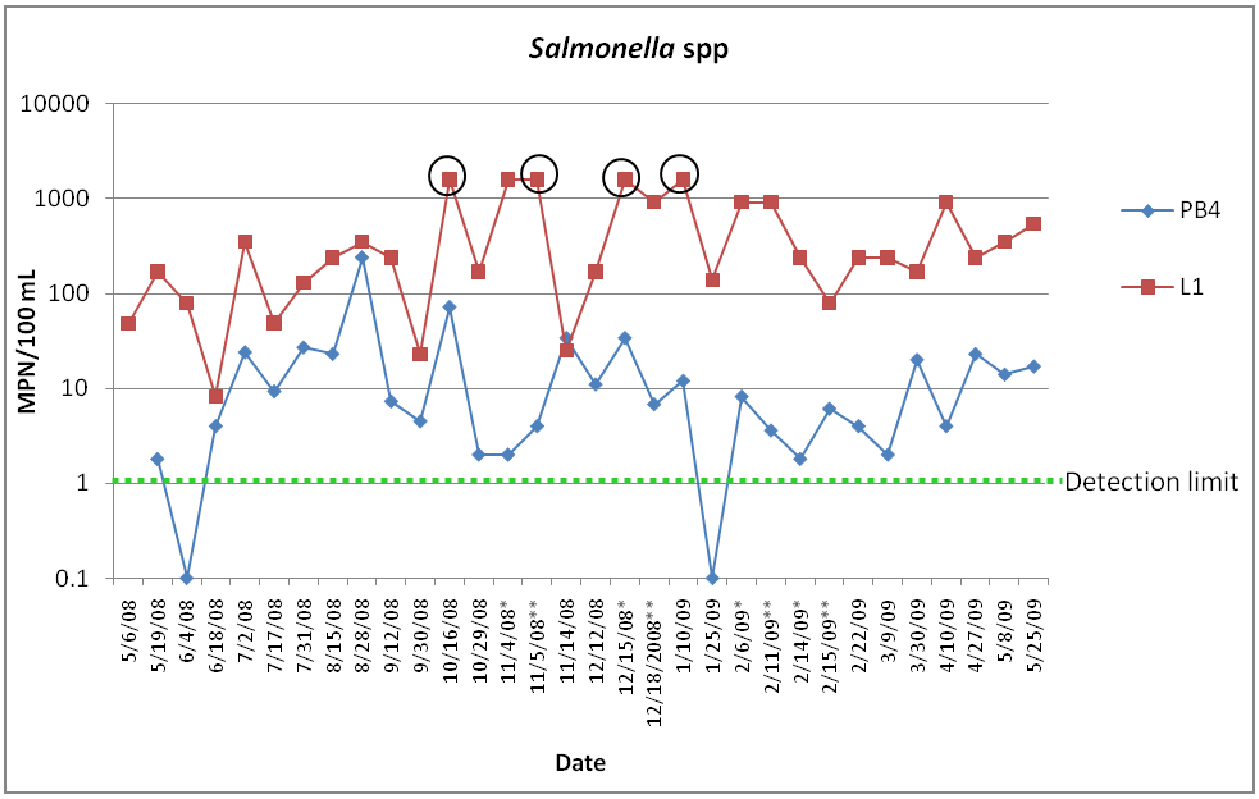


Figure 6.4.4.4-1. Cell count of *Salmonella* spp in PB4 and L1 from 5/6/2008 to 5/25/2009. All samples were collected during spring tides, during rain events (*) or immediately after rain events (**). Samples with undetectable levels of the pathogen were arbitrarily given a value close to zero and are shown below the detection limit (dotted line). Samples enclosed in a black circle exceeded the maximum detection threshold of the assay and were given the threshold value as estimation.

6.4.4.5. *Shigella* spp.

Shigella spp. detected in relatively low concentrations at both sites and exhibited a linked pattern in temporal shifts (Figure 6.4.4.5-1). At least three times, increases in *Shigella* spp. concentrations at L1 lead to similar increases in concentration at PB4, with a lag of two weeks to a month. In general, concentrations at L1 were higher than at PB4. The oral infective dose for *Shigella* spp. is low – as few as 10 cells could initiate an infection. Shigellosis has been associated with recreational water due to exposure to human excrement but not sewage contamination (Frank et al., 1988).

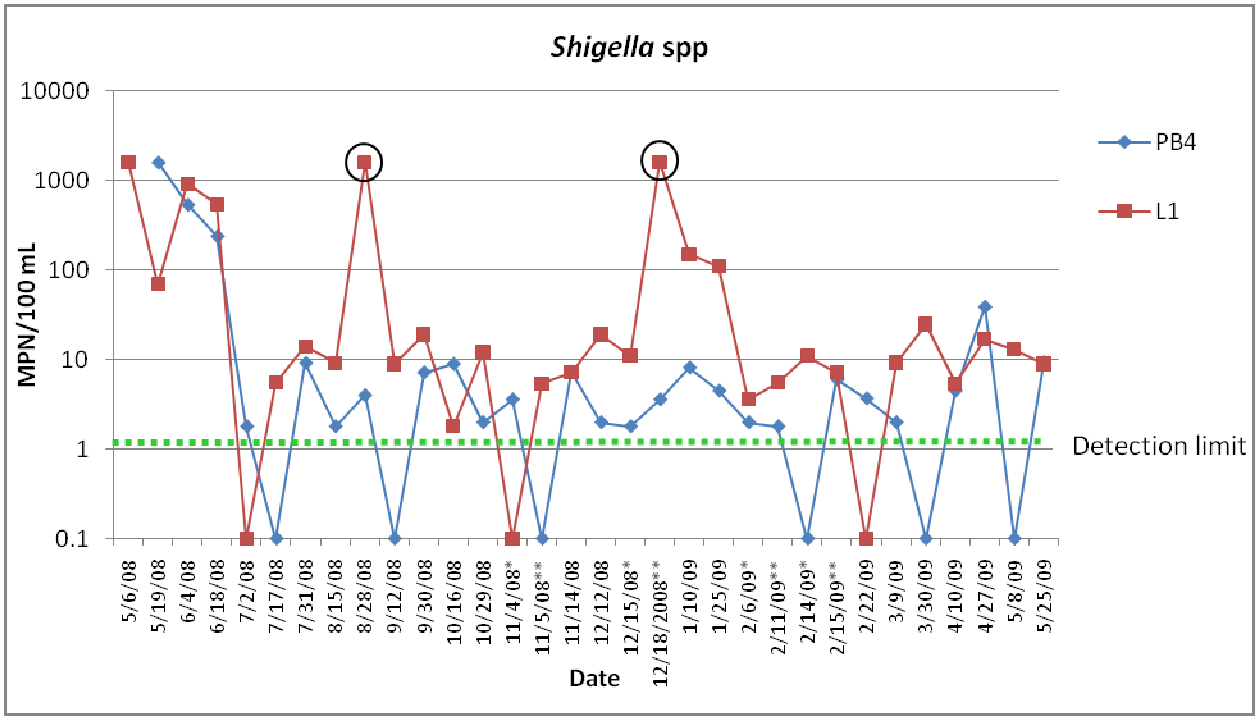


Figure 6.4.4.5-1. Cell count of *Shigella* spp in PB4 and L1 from 5/6/2008 to 5/25/2009. All samples were collected during spring tides, during rain events (*) or immediately after rain events (**). Samples with undetectable level of the pathogen were arbitrarily given a value close to zero and are shown below the detection limit (dotted line). Samples enclosed in a black circle exceeded the maximum detection threshold of the assay and were given the threshold value as estimation.

6.4.4.6. *Vibrio parahaemolyticus* and *V. vulnificus*

Both *Vibrio* spp. tested are naturally found in coastal environments worldwide and their prevalence is usually correlated with the water temperature. Indeed, the prevalence of *V. parahaemolyticus* in PB4 appeared lower during the winter season and rain events did not appear to have a significant effect (Figure 6.4.4.6-1). However, after statistical analyses, no correlation was found between water temperature and *V. parahaemolyticus* densities at this site, possibly due to the relatively high temperature at Pismo Beach – the lowest water temperature of all the sampling dates was only 8.9 ^{o}C. The oral infective dose for *V. parahaemolyticus* is high for healthy individuals (Yeung et al., 2004) but unknown (though presumably high) for *V. vulnificus*. However, serious illness such as septicemia can occur with less than 100 organisms for compromised individuals (FDA BBB).

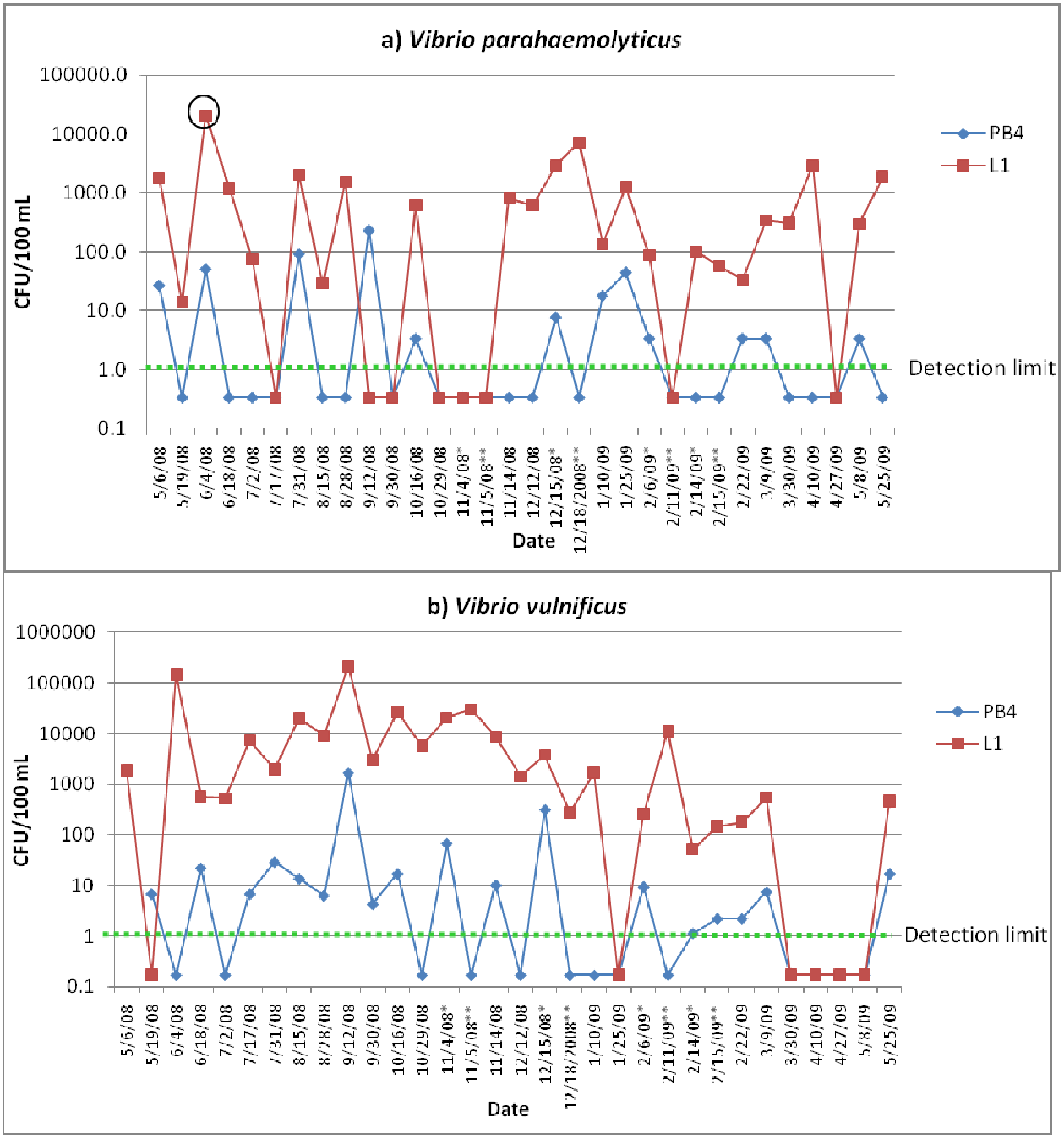


Figure 6.4.4.6-1. Cell count of (a) *V. parahaemolyticus* and (b) *V. vulnificus* in PB4 and L1 from 5/6/2008 to 5/25/2009. All samples were collected during spring tides, during rain events (*) or immediately after rain events (**). Samples with undetectable levels of the pathogen were arbitrarily given a value close to zero and are shown below the detection limit (dotted line). Samples enclosed in a black circle exceeded the maximum detection threshold of the assay and were given the threshold value as estimation.

6.4.4.7. Bacterial Pathogens in Pigeon Feces

Bacterial concentrations in feces could only be determined for three of the seven pathogens tested (Table 6.4.4.7-1). Upper bounds for pathogen concentrations were established based on detection limits for each test. Prevalence of *Campylobacter* spp. in pigeon feces was estimated by growth in enrichment media, followed by confirmation of *Campylobacter* spp. by growth on selective medium (see section 4.6), Gram stain, motility and microscopic inspection.

Table 6.4.4.7-1. Infective dose, concentration and prevalence data on pathogens in pigeon feces.

Pathogen	Infective Dose[a]	Concentration[b]	Presence
Aeromonas spp.	unknown	$1.3 \pm 0.9 \times 10^4$	85 %
Pseudomonas spp.	unknown	$2.7 \pm 1.5 \times 10^8$	83 %
Pseudomonas aeruginosa	$>10^3$	$< 10^2$	0
Salmonella spp.	10^3-10^5	$5.2 \pm 0.4 \times 10^4$	33 %
Campylobacter spp.	10^3-10^4	$< 10^3$	31 %
Vibrio parahaemolyticus	~10^6	$< 10^2$	0
Vibrio vulnificus	unknown	$< 10^2$	0

[a] Infective dose is the number of cells required to infect 50% of test subjects.
[b] CFU/gram feces. Error shown is standard deviation.
[c] Presence is the percentage of samples that yielded any growth of the specified organism.

6.4.5. Protozoan Pathogens

Data quality issues were experienced continuously for assays assessing the concentrations of the two protozoa, *Cryptosporidium* and *Giardia*. However, a subset of the samples collected was processed to generate results (Table 6.4.5-1). While some samplings clearly showed the presence of these parasites, the likelihood of false negatives cannot be overlooked because the percent recovery for the assay was very low. Even considering all the assay pitfalls, it appears that parasites were occasionally present at both sites. Since the infective dose of these parasites is very low, 1 to 10 organisms, there is an intermittent potential risk of infection through ingestion of water from PB4 or L1.

Table 6.4.5-1. Prevalence of *Cryptosporidium* and *Giardia* in10L water samples from PB4 and L1.

	Cryptosporidium		***Giardia***	
Date	**PB4**	**L1**	**PB4**	**L1**
19-May	–	–	–	–
4-Jun	+	–	–	+
18-Jun	–	+	–	–
2-Jul	–	+	++	–
17-Jul	++	++	+	++
31-Jul	–	–	–	–
15-Aug	–	+	–	–
28-Aug	–	+	–	–
29-Oct	+	–	+	–
14-Nov	–	–	–	–
3-Dec	+	+	–	++
12-Dec	–	++	–	–
15-Dec*	–	+	–	–
18-Dec**	–	–	–	–
Prevalence	29 %	57 %	21 %	21 %

+ indicates the number of organisms is between 1-10;
++ indicates >10 organisms.
* indicates samples taken during a rain event.
** indicates samples taken after a rain event.

6.4.6. *E. coli* Ribotype Matching to Massive Source Library

IEH returned ribotype matching results from a total of 675 *E. coli* strains isolated from water samples at Pismo Beach. From 0 to 4 *E. coli* strains were isolated and analyzed from each sample with an average 2.5 strains per sample. The ribotypes generated from these strains matched ribotypes in the IEH library from 33 different animal sources which were placed into 5 different categories to facilitate analysis (Table 6.4.6-1). Dog was the most common "Domestic" animal source, and bovine (cow) the most common "Livestock" source. A generic "avian" was the most common "WildBird" source while deer, raccoon and rodent were the common "WildMammal" sources. Several interesting and unexpected fecal source matches were returned, including bear, opossum, rabbit, chicken and poultry. It seems unlikely that these sources represent significant fecal contributions to the FIB counts at Pismo Beach. A total of 106 strains, 15.8%, did not produce a match and were categorized as "Unknown".

Table 6.4.6-1. Categories for the fecal source matches of *E. coli* strains sent to IEH for ribotyping. The number of strains in each match and category is listed in parentheses. "Unknown" indicates the number of strains, which did not match any ribotype in the IEH library. The "shellfish" source was placed in the "livestock" category for lack of a logical alternative.

Domestic (156)	Human (17)	Livestock (29)	WildBird (259)	WildMammal (108)
canine (15)	human (8)	bovine (18)	avian (142)	bear (1)
dog (117)	sewage (9)	chicken (1)	coot (6)	coyote (3)
cat (7)		horse (5)	crow (11)	deer (26)
feline (9)		poultry (3)	duck (9)	opossum (6)
feral cat (8)		shellfish (2)	egret (3)	porcine (10)
			goose (6)	rabbit (2)
			gull (47)	raccoon (36)
			pelican (1)	rodent (24)
			pigeon (16)	
			rock dove (13)	
			snowy egret (1)	
			spotted sandpiper (2)	
Unknown (106)			turnstone (2)	

Most samples sent to IEH were from the immediate vicinity of the pier (sites PB3.5 to PB4.5) except during rain events when all sites were sampled for IEH (Table 6.4.6-2). Across the entire sampling regime a total of 256 *E. coli* strains, 38%, matched some kind of "WildBird" source. The next highest source category was Domestic, accounting for 23% of the *E. coli* strains. The WildMammal source category accounted for 16%, Human sources accounted for 2.5% and Livestock sources accounted for 1.6% of all the *E. coli* strains. The distribution of sources was not different across the sampling sites. More specifically, the proportion of *E. coli* strains matching WildBird sources was not significantly different (Chi squared test $p = 0.3$) near the pier (sites PB4 and PB4.1 combined) compared to either north of the pier (sites PB4.2, PB4.5 and PB5 combined) or south of the pier (sites PB3.8, PB3.5and PB3 combined).

Table 6.4.6-2.

Site	Domestic	Human	Livestock	WildBird	WildMammal	Unknown	Total
PB1	3			2		2	7
PB2		2	1	2		1	6
PB3	5			6	2		13
PB3.5	29	2	6	45	18	11	111
PB3.8	29		4	40	19	26	118
PB4	25	6	4	53	30	31	149
PB4.1	19	2	4	33	16	5	79
PB4.2	20	2	4	31	8	12	77
PB4.5	13	1	3	27	6	9	59
PB5			1	6	3	2	12
O4.1	2	1		2	2		7
C1	1	1		1		1	4
C2	3			1	1	1	6
C3			1	6			7
L1	7		1	4	3	5	20
Total	156	17	29	259	108	106	675

The ribotyping FST method also confers the ability to track the frequency of collecting *E. coli* strains with the same ribotype, possibly indicating a similar origin. Amongst the 675 *E. coli* strains collected, there were 416 different ribotypes. While most ribotypes were collected only once in the study some were collected much more often, and this was not evenly distributed across the source categories (Figure 6.4.6-1). Some ribotypes in the WildBird and Domestic categories were collected more than seven times. Most interesting was a ribotype from a dog source that was collected 76 times. This dog ribotype was only collected on the beach from PB3 to PB4.5, most commonly just south of the pier, and appeared throughout the summer of 2008 from May through August. This could represent either an *E. coli* strain that is very common among dogs that visit Pismo Beach, or it could represent feces from one or more dogs that often defecated on the beach in the summer of 2008. Another interesting ribotype from a pigeon source was collected 12 times. This ribotype was only collected from sites within 50 to 150 meters of the pier (PB3.5 to PB4.2) in June 2008 and August 2008.

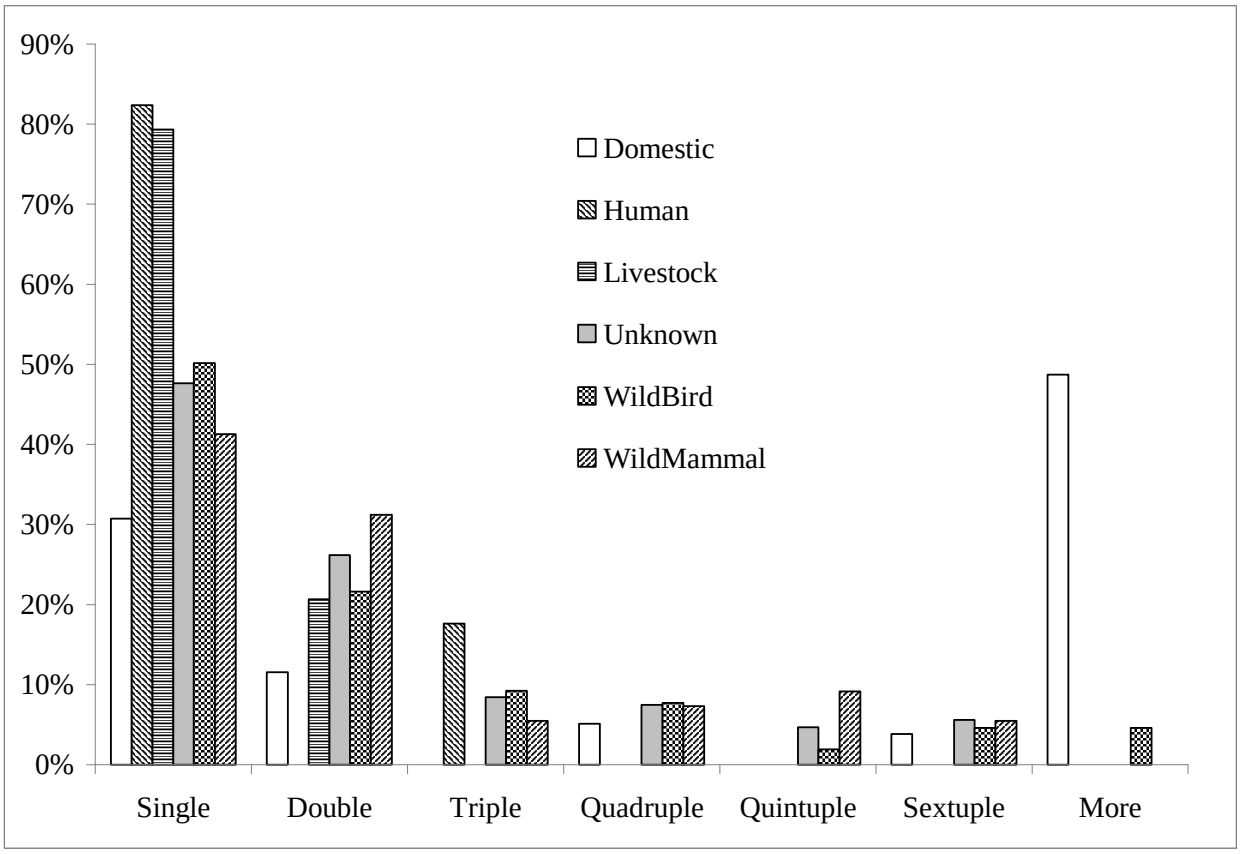


Figure 6.4.6-1. Distribution of ribotype frequency by source category. "Single" indicates a ribotype collected only once in the study, "Double" indicates strains collected twice, and so on.

6.4.7. Rapid Human *Bacteroides* Detection on Digital PCR Device

ALL, in North Carolina, subcontracted with the EBI to test the use of a hand-held digital PCR device for the rapid detection of human feces in seawater. In the initial assays, various dilutions of sewage in seawater were loaded directly into a digital microfluidics cartridge. For this assay, DNA extraction was performed using the Ademtech™ (D-N-Adem™ for Gram Positive and Gram Negative Bacteria) magnetic bead DNA extraction kit. This kit includes DNA-binding magnetic beads and a lysis buffer suitable for DNA extraction from bacteria. Seawater samples mixed with raw sewage from the Pismo Beach wastewater facility were serially diluted 10-fold, generating 3 concentrations of sewage (10^{-1}, 10^{-2} and 10^{-3}). Undiluted, clean seawater was used as a negative control. DNA extraction and qPCR were both performed on the hand-held device. Only the 10^{-1} polluted seawater dilution produced a signal by qPCR with C_T values of approximately 31 cycles (Figure 6.4.7-1). Native seawater (negative control) and other dilutions did not yield a signal during qPCR on the hand-held device (data not shown).

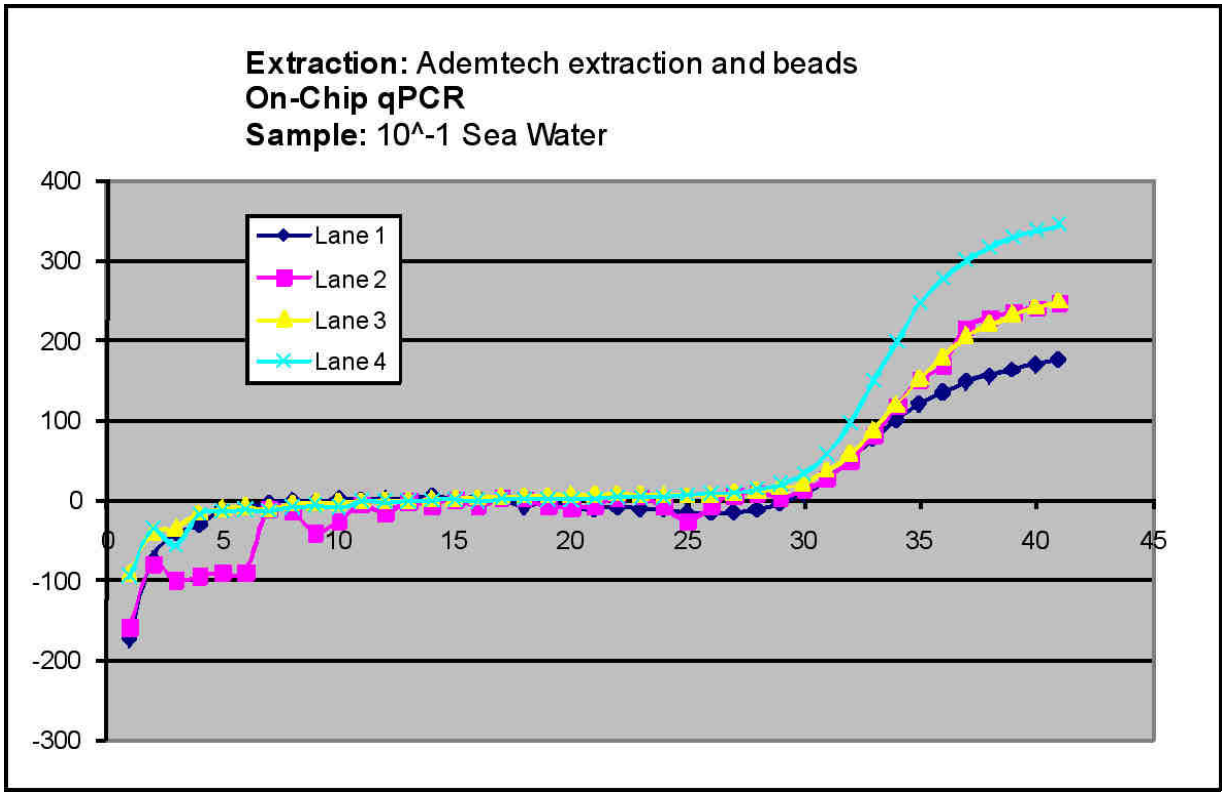


Figure 6.4.7-1. Detection of Human *Bacteroides* DNA via PCR on ALL's hand held device (on-chip). Four samples of a 10^{-1} dilution of sewage in seawater were run simultaneously.

Further assay optimization was performed using a bench-top protocol for qPCR and DNA extraction. Bench-top experiments were modified from the initial experiments on the hand-held device in several ways. First, the magnetic beads provided in the Ademtech kit did not appear to respond very strongly to the magnets used on our digital microfluidics instrument. We therefore replaced them with ChargeSwitch™ (Invitrogen®) beads, another brand of DNA capture beads which we have extensive experience with, and which respond very strongly to the magnets on our instrument. However, the Ademtech lysis buffer was still used for extraction. We added an additional variable to this procedure by reducing the amount of beads employed for DNA extraction. To the initial 100 µL sample, beads were added at 40, 10, 5, and 1 µL. By utilizing the fewest number of beads possible, our elution volumes will be minimized. This will be helpful for later translation to an assay on the hand-held device.

Starting samples, 100 µL of 10^{-1} dilution of sewage, were used for these experiments. Extraction was performed with various volumes of ChargeSwitch beads, using the Ademtech lysis buffer. Following extraction, qPCR was performed on our IQ5 instrument using identical primers, times and temperatures compared to assays performed on the hand-held device (Figure 6.4.7-2). The ChargeSwitch beads, which in our experience translate well to assays on the hand-held device, appear to function equally well when 40 µL or 1 µL of beads are used. This will allow us to use a minimal volume of elution buffer, and generate a very high percent yield of extracted DNA.

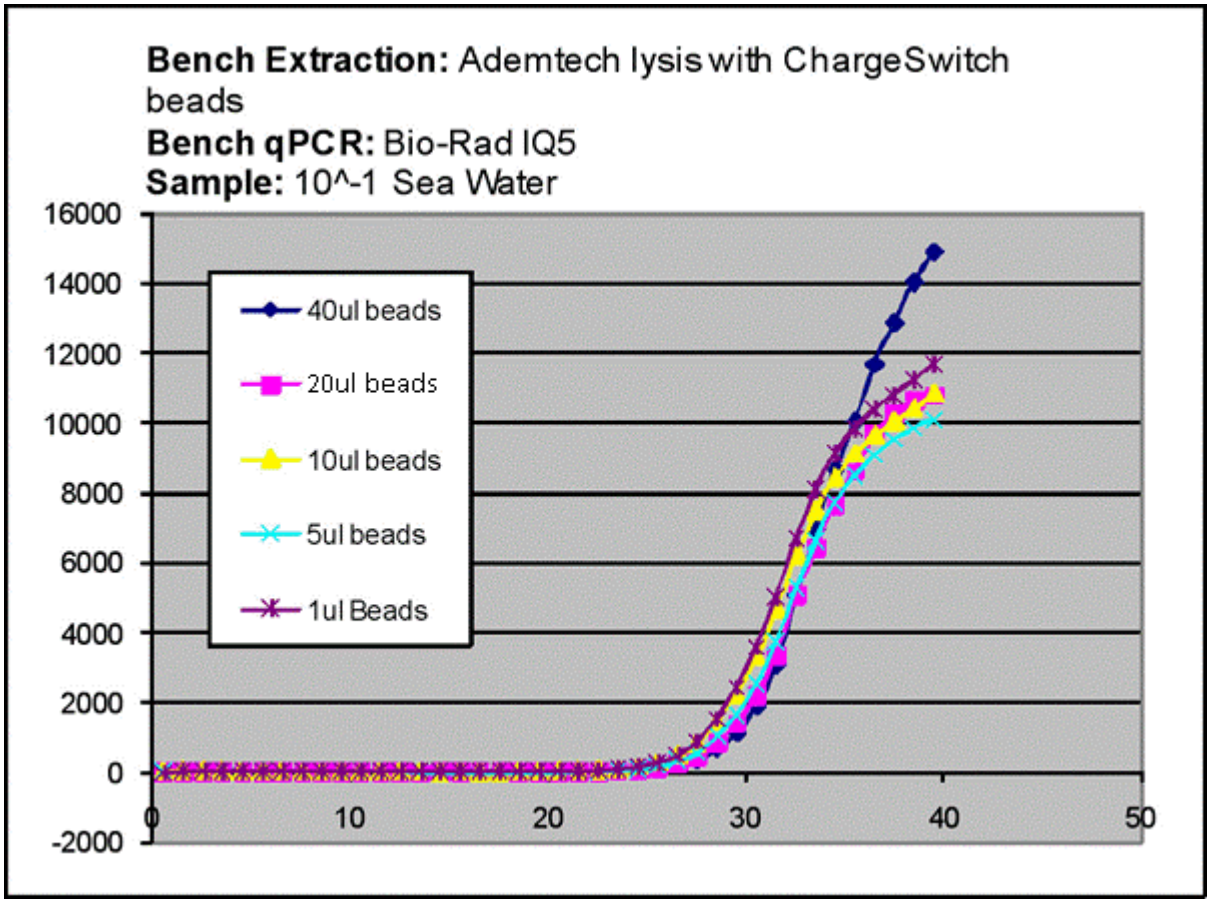


Figure 6.4.7-2. Combining Ademtech lysis buffer and ChargeSwitch™ beads greatly enhances DNA extraction. ChargeSwitch™ beads also appear to provide adequate DNA binding capacity when only 1 µL is used.

In our final experiment, we again examined serial dilutions of sewage in seawater, in an effort to observe a titration curve during qPCR commensurate with the dilutions (Figure 6.4.7-3). The same protocol developed above was used here: combining Ademtech lysis buffer with ChargeSwitch™ beads for DNA extraction, and performing qPCR on our Bio-Rad® IQ5™. Diluted sewage gave results consistent with expectations, where DNA product was amplified from all three dilutions, and in the expected rank order. Sewage diluted 10^{-1} showed amplified product with a C_T of 29, which is an improvement over the previous experiments. Other dilutions gave C_Ts of 36 (10^{-2}) and 38 (10^{-3}). Native seawater did not show amplification (non-specific C_T of 41 which is typical in negative controls). These data suggest that our protocol is valid for the extraction and amplification of Human *Bacteroides* DNA from sewage diluted in seawater.

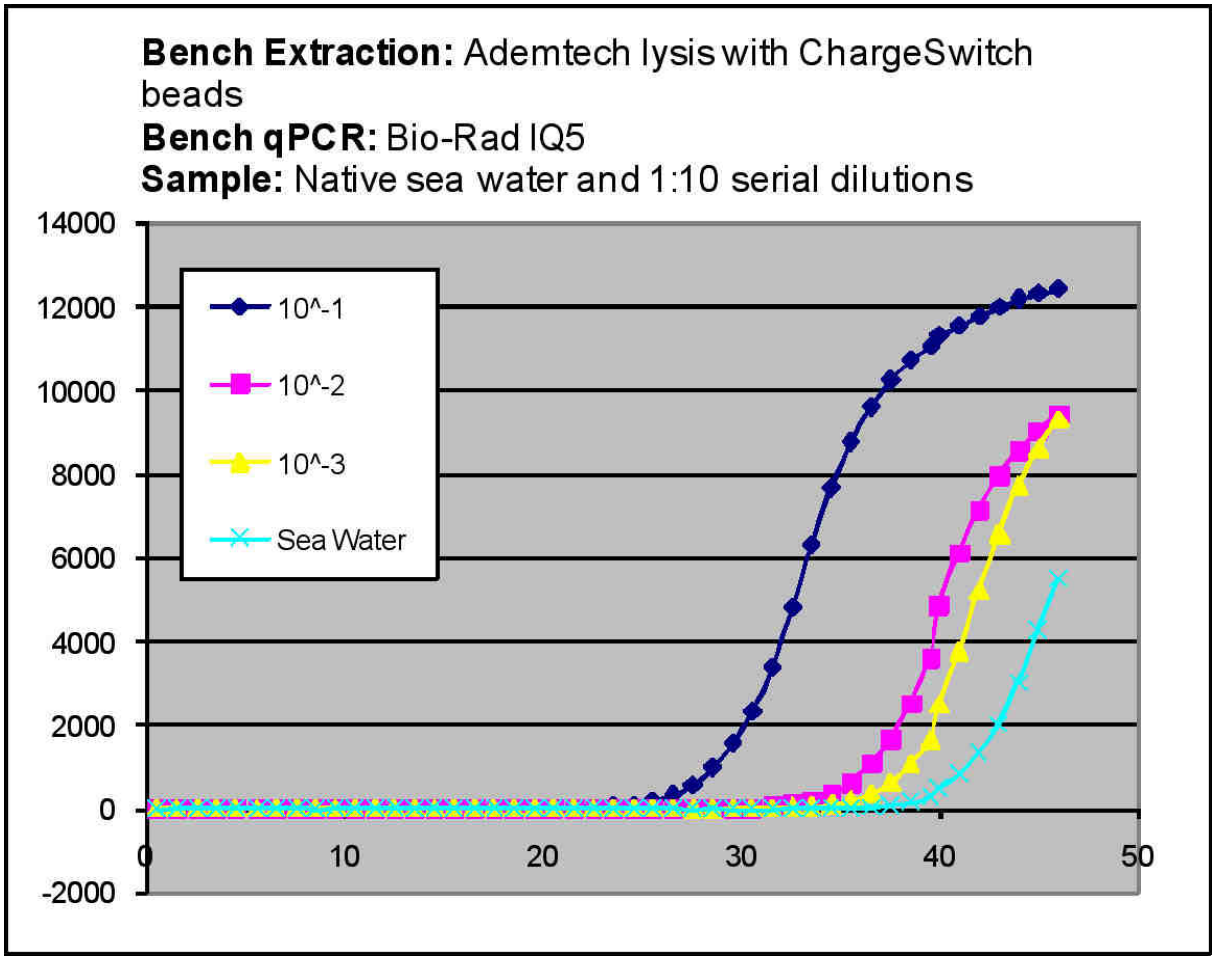


Figure 6.4.7-3. Combining Ademtech lysis buffer and ChargeSwitch™ beads greatly enhances DNA extraction and yields signals in the correct rank order expected by serial dilution inputs.

We also attempted to translate these assays to our digital microfluidic format on the hand-held device. After performing several experiments using the methods describe above, detection of Human *Bacteroides* was only accomplished at the 10^{-1} dilution sample. PCR from other samples did not reveal any *Bacteroides* contamination, suggesting the current assay is not sufficiently sensitive for these concentrations. To improve the assay we undertook several improvements. First, we obtained a fresh raw sewage sample from a local wastewater treatment facility in case the Pismo Beach sewage samples had aged to the point of signal loss. Next, we added a mechanical lysis step to allow us to use more standardized reagent concentrations for additional steps of the process. Then we increased the sample size to increase the total *Bacteroides* population entering processing. Last, instead of washing the DNA-bound Ademtech beads twice, we washed four times. This has resulted in earlier C_Ts on other qPCR experiments.

These improvements were employed to generate a new protocol for the isolation of DNA prior to qPCR on the hand-held device. First glass beads were added to 500 µL of raw sewage and shaken mechanically to perform the initial lysis step. This sample was then diluted in commercial-grade sea water (Sigma) at 10^{-1} and 10^{-2}. Samples were then Proteinase K and RNAse A treated for 5 min. 100 µL of each dilution was then added to the equivalent of 0.34

mL of Ademtech DNA-binding beads. Beads were then washed four times in wash buffer. The bead pellet was then brought back up in 3.2 µL of PCR mastermix containing universal *Bacteroides* primers (not specific for a given host species). PCR was then performed on-chip. Dilutions of 10^{-1} and 10^{-2} exhibited C_T values of approximately 20 and 24 cycles respectively. Additional dilutions will be run in subsequent experiments to determine the ability of the current system to detect *Bacteroides* at these concentrations.

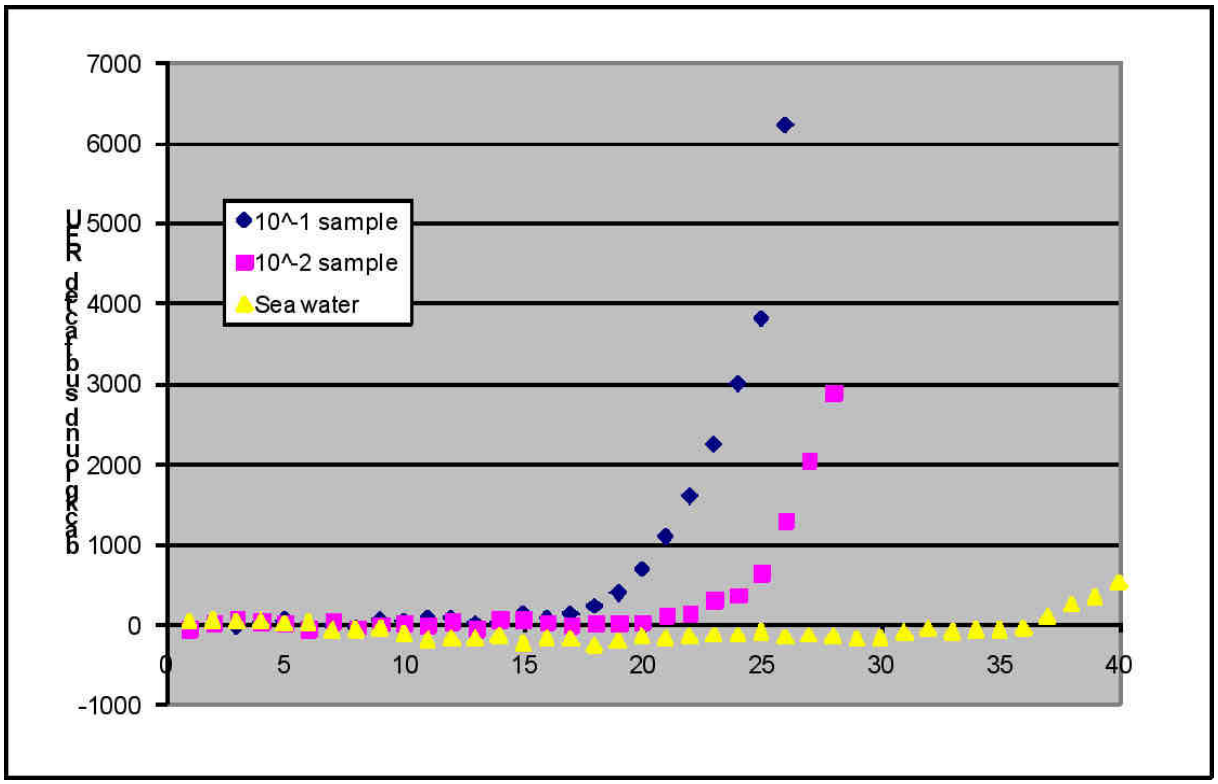


Figure 6.4.7-4. qPCR on the hand-held device using modified extraction and DNA isolation protocol.

The modified protocol provided excellent preliminary results (figure 6.4.7-4), and we are eager to continue to examine further dilutions of *Bacteroides* using qPCR on the hand-held device. We are also interested in fully automating the DNA extraction and isolation procedures on the hand-held device, to provide optimal ease of use. Future activities toward accomplishing this goal would include: performing the same procedure with more dilute samples; repeating these experiments with fresh seawater samples; automating extraction on chip using a mechanical lysis method such as sonication; and obtaining standard concentrations of *Bacteroides* to quantitatively determine the limit of detection for the current system.

We are eager to continue this program, and generate a more fully automated system to generate *Bacteroides* concentration data for seawater samples.

6.5. Volunteer Beach Survey Results

Volunteers walked a 600 m transect from PB5 north of the pier to PB3 in the south. Feces sighted within a 2 m wide path were counted into four zones: from PB5 to PB4.2; from PB4.2 to

directly under the middle of the pier; from the middle of the pier to PB3.8; and from PB3.8 to PB3. These counts took approximately 30 minutes and were taken once a day during the same two months when daily water samples were being collected from 6/26/2008 to 8/25/2008. Volunteers showed up when they had time so count times varied from 6 am to 5 pm. On average began at 9 am, very close to the time water samples were being taken. Diapers were noted on the beach, off the transect path, on 6/26/2008, 6/28/2008, and 8/20/2008. Other interesting observations included large flocks of sea birds feeding just off shore on 7/19/2008, 7/29/2008, and 8/25/2008, and dead birds on the beach on five occasions. By far the highest count of bird droppings were seen close to the pier (Table 6.5-1). Dog droppings were rarely seen on the transect.

Table 6.5-1. Feces counts along the Pismo Beach volunteer transect. Average counts over 61 days (standard deviation in parentheses) along with total days fecal type was observed on the transect path (percent of total days in parentheses). Dog droppings were twice noted off the transect path (data not included).

Count Zone	Bird droppings seen per day	Days when bird dropping were seen	Dog droppings seen per day	Days when dog dropping were seen
PB5 to PB4.2	21 (31)	57 (93%)	0.10 (0.35)	5 (8%)
PB4.2 to Mid Pier	141 (92)	61 (100%)	0.02 (0.13)	1 (2%)
Mid Pier to PB3.8	81 (44)	61 (100%)	0.00 (0.00)	0 (0%)
PB3.8 to PB3	10 (22)	57 (93%)	0.10 (0.35)	5 (8%)

After walking the transect path, volunteers went on top of the pier and observed the beach in both directions for 30 minutes, counting people and dogs and noting activities. Specific sets of observations were mandated (Table 6.5-2) and other notable activities were also written down. One recurring activity noted was lifeguard trainings on 7/2/2008, 7/11/2008, 7/21/2008 and 8/7/2008. A one-time survey at the pier estimated the size of the Pismo pigeon flock at 459 birds with 218 pigeon nests on the structural members of the pier itself. The numbers of dogs and people noted each day during this 30 minute period varied wildly and the average per day was not significantly different across the days of the week (data not shown).

Table 6.5-2. Volunteer observations over a 30-minute period for 61 consecutive days (standard deviations or percentage of total are presented in parentheses).

Observation	Average per day	Days observed	Totals
Dogs on the beach	9.3 (4.8)	61 (100%)	566
Dogs seen defecating	0.23 (0.59)	10 (16%)	14
Dog droppings picked up	0.18 (0.43)	10 (16%)	11
People on the beach	197 (178)	61 (100%)	12,039
Kids in diapers	2.9 (5.4)	28 (46%)	175
People feeding birds	0.03 (0.2)	2 (3%)	2
Horses on the beach	0.1 (0.7)	3 (5%)	9

6.6. Integrated Results

Several large data sets were collected in this study. This section is concerned with putting some of the separate data together into a more comprehensive whole.

6.6.1. Statistical Models for Predicting FIB Levels

General linear models were used to investigate the relationship between log transformed FIB counts (TC, *E. Coli*, and Ent) and environmental and physical variables [MSL = mean sea level (feet), Wash = calculated time since the tide was last as high as present (hours), Rain = total daily precipitation (inches), Turb = turbidity (Nephelometric Turbidity Units), Sal = salinity (millisiemens/centimeter), UV = absorbance at 254 nanometers (absorbance), WindX = onshore wind speed (meters/second), WindY = alongshore wind speed (meters/second), CurX = onshore surface currents (meters/second), CurY = alongshore surface currents (meters/second), Hm0 = significant wave height (meters), Tm02 =mean wave period (seconds), Mdir = mean direction from which waves are coming[1] (degrees centigrade), WE = weekend indicator, and Site] for the PB1 through PB5 beach sites (Table 6.6.1-1).

Intermediate models considered several interactions including Wash:MSL, Wash:Site, MSL:Site, Mdir:Site, CurX:Site, CurY:Site, and Rain:Turbidity. Partial F-tests demonstrated that collectively the Wash:MSL, Wash:Site, and MSL:Site interactions provided no model utility for any fecal indicator and thus for parsimony were dropped from subsequent consideration [$F(18,380) < 1.361$, $p > 0.148$]; the remaining interactions were retained in the final model (Table 6.6.1-2).

[1] While this variable is circular in nature, no Cartesian decomposition was necessary as the degree range was narrow and did not span due north (0 degrees).

To investigate the possible association between Human and/or Dog *Bacteroides* and FIB, binary *Bacteroides* indicator variables were added as predictors to the aforementioned (reduced) model and their collective significance tested using partial F-tests. As no significant association was found the *Bacteroides* data was dropped from subsequent analysis [$F(18) < 1.51$, $p > 0.08$].

Residuals from the final model demonstrated reasonable Gaussian behavior with stable variance. While some data points exhibited high leverage (Cook's distance), removal of these points did not substantially alter model conclusions (term sign or significance).

This statistical model used to investigate relationships between physical and environmental variables and FIB counts only considered data collected at Pismo Beach sites between 5/6/2008 through 5/25/2009 since the summer of 2007 was considered preliminary data. Only records with complete observations for all variables in the model were included leaving 432 of 2,043 records for analysis (384 records occurred prior to 5/6/2008, 209 records were missing time of day, 1,141 were missing Salinity, 989 were missing Turbidity, 1,388 were missing wind speed, and 624 missing AWAC data).

Table 6.6.1-1. Coefficients, t-values and p-values for the variables included in the final general linear model for predicting FIB counts. Each test had 380 degrees of freedom. Significant variables are denoted by boldface type p-values. Rain:Turb refers to the interaction between variables Rain and Turb.

	TC			*E. coli*			Ent		
Variable	**Coef**	**t**	**p**	**Coef**	**t**	**p**	**Coef**	**t**	**p**
Wash	0.034	5.229	**0.000**	0.038	5.749	**0.000**	0.028	4.921	**0.000**
MSL	0.036	1.407	0.160	0.038	1.440	0.151	0.095	4.108	**0.000**
WE	-0.054	-0.951	0.342	-0.053	-0.920	0.358	0.033	0.649	0.517
Rain	-1.402	-0.963	0.336	-1.996	-1.359	0.175	-1.275	-0.979	0.328
Turb	-0.022	-0.765	0.445	-0.021	-0.697	0.486	-0.012	-0.474	0.636
Sal	0.024	3.230	**0.001**	0.022	2.970	**0.003**	0.008	1.256	0.210
UV254	-0.679	-0.843	0.400	-0.304	-0.375	0.708	-0.772	-1.073	0.284
WindX	0.016	0.909	0.364	0.042	2.358	**0.019**	0.036	2.285	**0.023**
WindY	-0.013	-0.592	0.554	0.006	0.276	0.783	0.014	0.672	0.502
Hm0	-0.228	-1.567	0.118	-0.473	-3.224	**0.001**	-0.090	-0.692	0.489
Tm02	-0.196	-4.929	**0.000**	-0.155	-3.861	**0.000**	-0.050	-1.406	0.160
Rain:Turb	0.476	1.563	0.119	0.316	1.027	0.305	0.703	2.582	**0.010**

Wave period (Tm02) and wave height (Hm0) were both significant predictors of *E. coli* levels, though only wave period helped predict TC counts (Table 6.6.1-1). The time since a tide was last this high (Wash) was also significant. Rain and Turbidity (through their interaction) are significant predictors of Ent counts as is sea level. Onshore wind (WindX) was a significant predictor for both *E. coli* and Ent counts, while salinity (Sal) was significant for both TC and *E. coli*. Time since the tide was last this high (Wash) was the only variable that was consistently a significant predictor for all three FIB counts. This variable appears to indicate that the longer it has been since a section of beach was washed by the tide, the more likely it is for more feces to have been deposited there (Figure 6.6.1-1).

Table 6.6.1-2. Coefficients, F values and p-values for variable groups and interactions in the final general linear model for predicting FIB counts (df = degrees of freedom). Significant variables are identified by boldface p-values.

		TC		***E. coli***		**Ent**	
Parital F-tests	**df**	**F**	**p**	**F**	**p**	**F**	**p**
Site:Mdir	9	6.590	**0.000**	9.455	**0.000**	3.101	**0.001**
All surface current	20	3.497	**0.000**	3.734	**0.000**	1.558	0.060
Surface current interactions	18	2.969	**0.000**	3.353	**0.000**	1.061	0.390
Site:CurX	9	1.910	**0.049**	2.879	**0.003**	0.846	0.574
Site:CurY	9	5.260	**0.000**	5.922	**0.000**	1.931	**0.046**
Wind	2	1.686	0.187	4.257	**0.015**	3.243	**0.040**
Rain, Rain:Turbidity	2	1.649	0.194	1.000	0.369	7.404	**0.001**

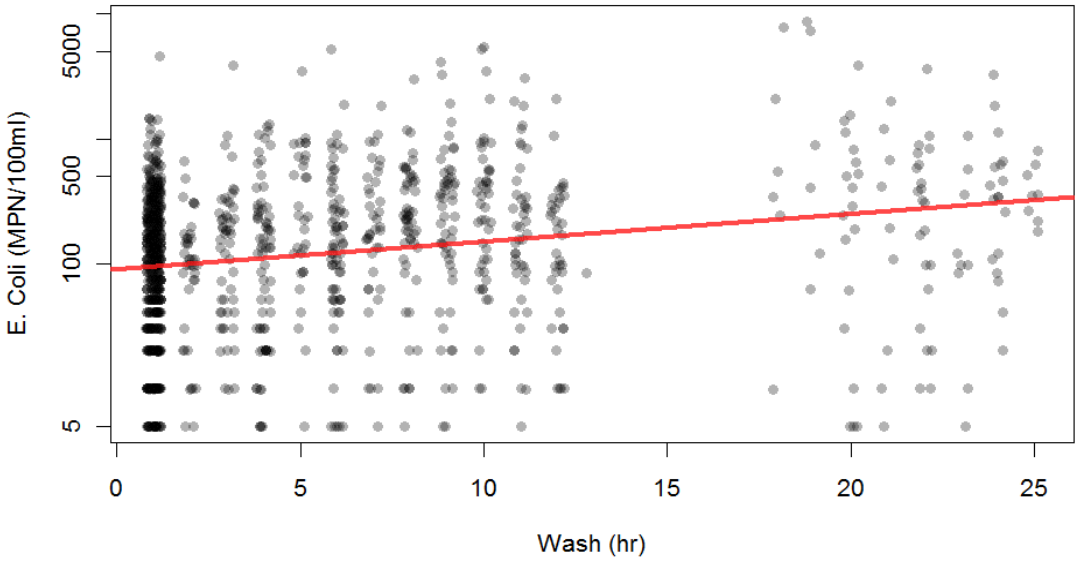


Figure 6.6.1-1. Visualizing and comparing the effect of Wash as a predictor of MPN values for *E.coli*. As time since the last tidal wash increases, so do the *E. coli* counts found in the sample.

Intriguingly, both ocean current and mean wave direction (CurX, CurY, Mdir) interacted with site for significant predictions of FIB counts (Table 6.6.1-2). Graphical analysis of the site interactions revealed some interesting relationships. For example, when wave energy came from the north (forcing water south along the beach) sampling sites to the north of the pier showed decreases in *E. coli*, while sites to the south of the pier showed increases (Figure 6.6.1-2). The inverse was true when waves approached the beach from the south. This is our best evidence that the most common and abundant source of fecal contamination at Pismo Beach is in the general area of the pier itself. Although we only display this effect for *E. coli*, it remained true for all three FIB counts.

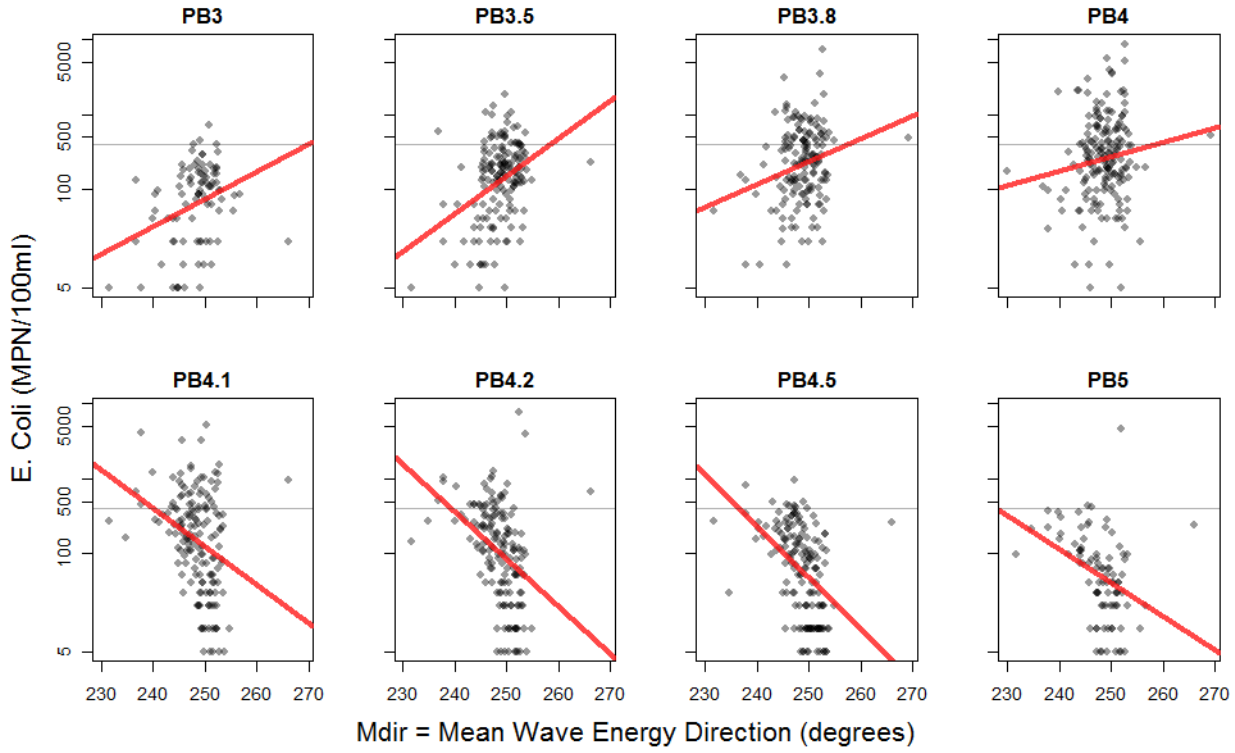
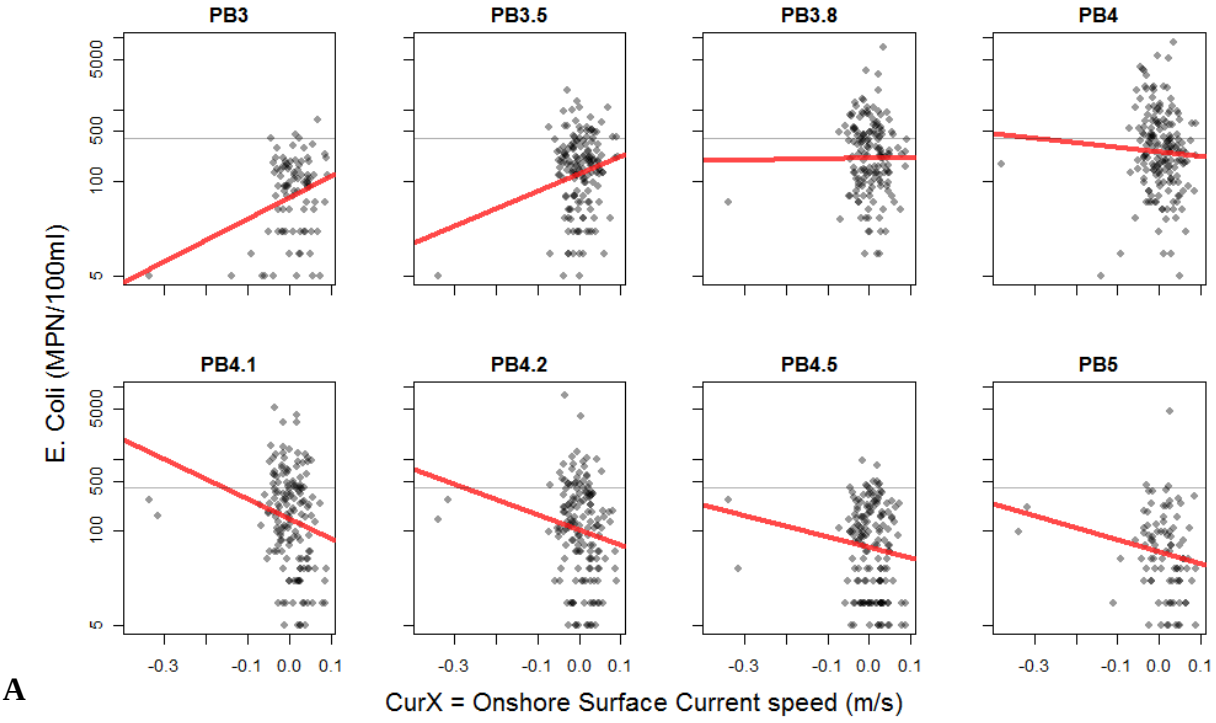
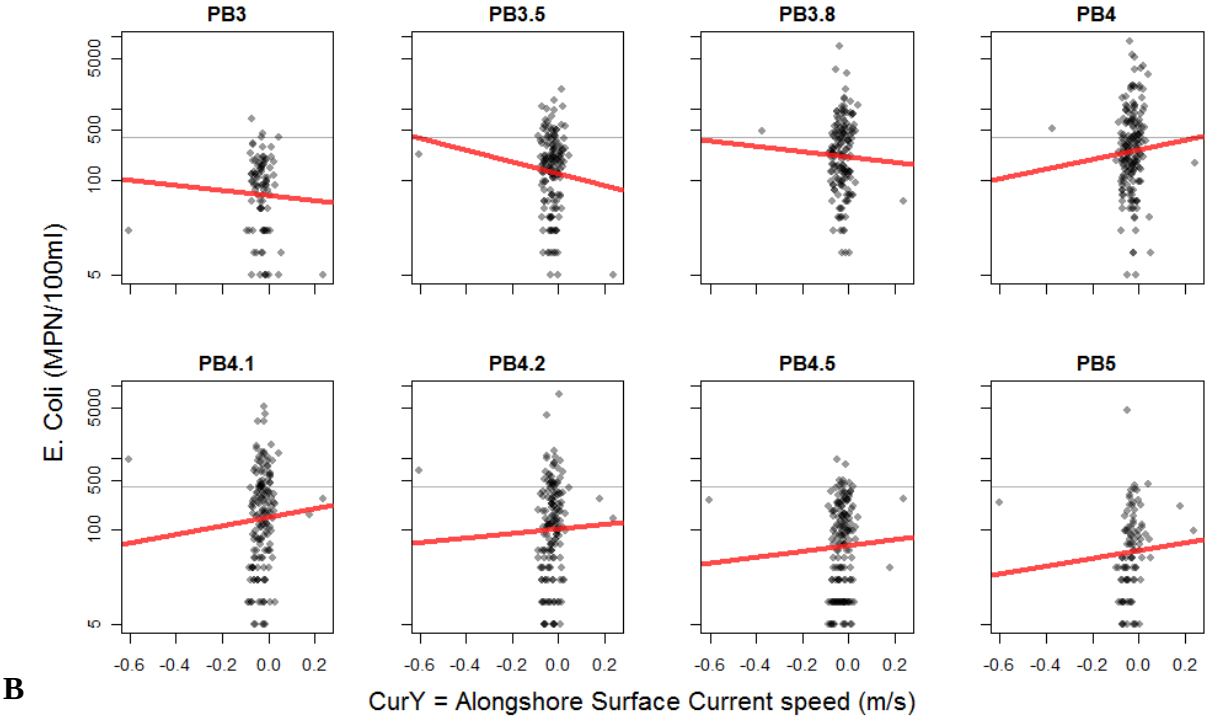


Figure 6.6.1-2. Visualizing and comparing the effect of wave direction (Mdir) as a predictor of MPN values for *E.coli* across sites PB3-PB5. An angle of approximately 244 degrees is perpendicular to the beach at the pier.

Site interactions with ocean currents are a little less clear but follow the same trend. Just like the effect of wave direction, when the ocean current moved water to the south of the pier (negative CurY), sampling sites south of the pier saw an increase in *E. coli* counts, and vice versa (Figure 6.6.1-3B). However, sites to the south of the pier saw an increase in *E. coli* counts with increasing onshore current (positive CurX) while sites to the north showed decreasing *E. coli* with increasing onshore current. This is confusing at first, but a quick reference to Figure 6.2.3-1 shows that CurX and CurY were highly correlated at the times FIB samples were taken. In fact, postitive CurX was almost always associated with a negative CurY. Thus, an onshore current also pushed water to the south resulting in higher counts south of the pier.



A



B

Figure 6.6.1-3. Visualizing and comparing the effect of Current (CurX in panelA, CurY in panel B) as a predictor of MPN values for *E.coli* across sites PB3-PB5. On shore current is positive when water is moving toward the beach. Alongshore current is positive when water is moving northward along the angle of the beach.

6.6.2. Comparison to FIB Data from Huntington Beach

Because the variables Wash and MSL seem to indicate that much of the source of fecal contamination at Pismo Beach comes from deposition of feces on the beach between tides, we analyzed historical data sets from the SLO-CPDH weekly samplings and from Huntington Beach. The SLO-CPDH data was made available via email and the Huntington Beach data was provided via email/ftp by George Robertson, Senior Scientist, Orange County Sanitation District. Huntington Beach tide data was approximated using the nearest available NOAA data for the Long Beach Terminal Island station. Furthermore, FIB counts were measured using membrane filtration methods

To enable direct comparison with our final model for Pismo Beach data, similar general linear models were fit to the historic Pismo (sites PB3–PB5, 2005–2007) and Huntington Beach (sites 27S–27N, 2001–2006) available data. Log transformed FIB counts were regressed on Site, Wash and MSL as well as all two-way interactions. Because these interactions were not significant in our primary model and only marginally significant for some sites at Huntington Beach, the interactions were dropped for the final model. For direct comparison, the above simple model was also fit to the current Pismo Beach data.

Residuals from the final models using the Pismo Beach data demonstrated reasonable Gaussian behavior with stable variance; however, the Huntington Beach residuals did not display Gaussian behavior and residual variance was only marginally stabilized. Due to the extremely large number of daily Huntington Beach observations and the fact that our models are not used for FIB prediction, but rather association, the lack of normality is not of concern.

Site was a significant predictor for all thee studies ($p < 0.001$). However, Wash and MSL were significant only for this study and the Huntington Beach study (Table 6.6.2-1). This brings up an interesting issue with regard to beach sampling. The Pismo Beach historic data from SLO-CPHD was collected once a week for two years at approximately the same time. Conversely, both the Huntington Beach study and this study incorporated sampling schemes designed to collect samples across a wide range of tidal scenarios (Rosenfeld et al., 2006). Thus, it seems likely that the failure to see a tidal effect with the historical data from Pismo Beach is due to either a sampling artifact or lack of power due to a much smaller sample size.

Table 6.6.2-1. Coefficient estimates, t-values and p-values for Wash and MSL as predictors of FIB counts in three studies (df = degrees of freedom). Significant p-values are denoted by boldface type.

			Wash			MSL		
Study	**FIB**	**df**	**Coef**	**t**	**p**	**Coef**	**t**	**p**
Huntington 2001-2006	TC	21110	0.003	4.138	**0.000**	0.031	6.278	**0.000**
	E. coli	21110	0.003	5.271	**0.000**	0.019	5.376	**0.000**
	Ent	21110	0.007	14.209	**0.000**	0.037	12.623	**0.000**
Pismo 2005-2007	TC	256	0.000	-0.446	0.656	-0.020	-0.719	0.473
	E. coli	256	0.000	-0.004	0.997	-0.031	-1.360	0.175
	Ent	256	0.000	0.192	0.848	-0.003	-0.197	0.844
This Study	TC	1164	0.001	2.636	**0.009**	0.034	3.508	**0.000**
	E. coli	1164	0.001	2.958	**0.003**	0.042	4.317	**0.000**
	Ent	1164	0.001	2.636	**0.009**	0.034	3.508	**0.000**

6.6.3. Statistical Models for predicting *Bacteroides* presence

Generalized linear models (logistic regression) were used to investigate the relationship between the presence/absence of *Bacteroides* (separate models for Human and Dog) and environmental/other variables [Rain = precipitation (inches), Wash = hours since the tide was last this high (hours), MSL = mean sea level (feet), Site, and Day = day of the week] for the PB3 through PB5 beach sites. Cow and Horse *Bacteroides* results were not modeled.

Intermediate models also included the presence/absence of one type of *Bacteroides* as a predictor of the other in addition to the aforementioned variables. Regardless of ordering (Dog or Human as the response), neither *Bacteroides* was a significant predictor of the other (p = 0.2633, 0.2862 for Human and Dog as predictors respectively). They were therefore dropped from models to allow for direct comparison of environmental variables between models.

Site and Rain had no significant association with the presence of either Human or Dog *Bacteroides* in the samples tested (Table 6.6.3-1). However, the day of the week was a significant predictor for both types of *Bacteroides*. In contrast, sea level was significant for Human *Bacteroides* only and Wash (time since the tide was last as high) was significant only for Dog *Bacteroides*.

Table 6.6.3-1. Coefficients, Chi-square values and p-values for the variables included in the final model for predicting the PCR amplification of human- and dog-specific *Bacteroides* (df = degrees of freedom). Significant p-values are denoted by boldface type.

		Human (residual df = 605)			Dog (residual df = 605)		
Variable	**df**	**coef**	**Chi-sq**	**p**	**coef**	**Chi-sq**	**p**
Site	7	.	7.719	0.358	.	7.352	0.393
Rain	1	-3.304	0.489	0.484	-3.900	1.050	0.305
Wash	1	0.026	1.167	0.280	-0.065	5.146	**0.023**
MSL	1	-0.502	16.725	**0.000**	0.164	1.851	0.174
Day of week	6	.	26.921	**0.000**	.	19.132	**0.004**

Graphical analyses showed that while the presence of both Human and Dog *Bacteroides* was significantly predicted by the day of the week, Human *Bacteroides* were most common on Monday through Wednesday, while Dog *Bacteroides* were more common Friday through Sunday (Figure 6.6.3-1). Human *Bacteroides* were more often found at lower tides and Dog *Bacteroides* were more common when it was less than 10 hrs since the tide was last this high.

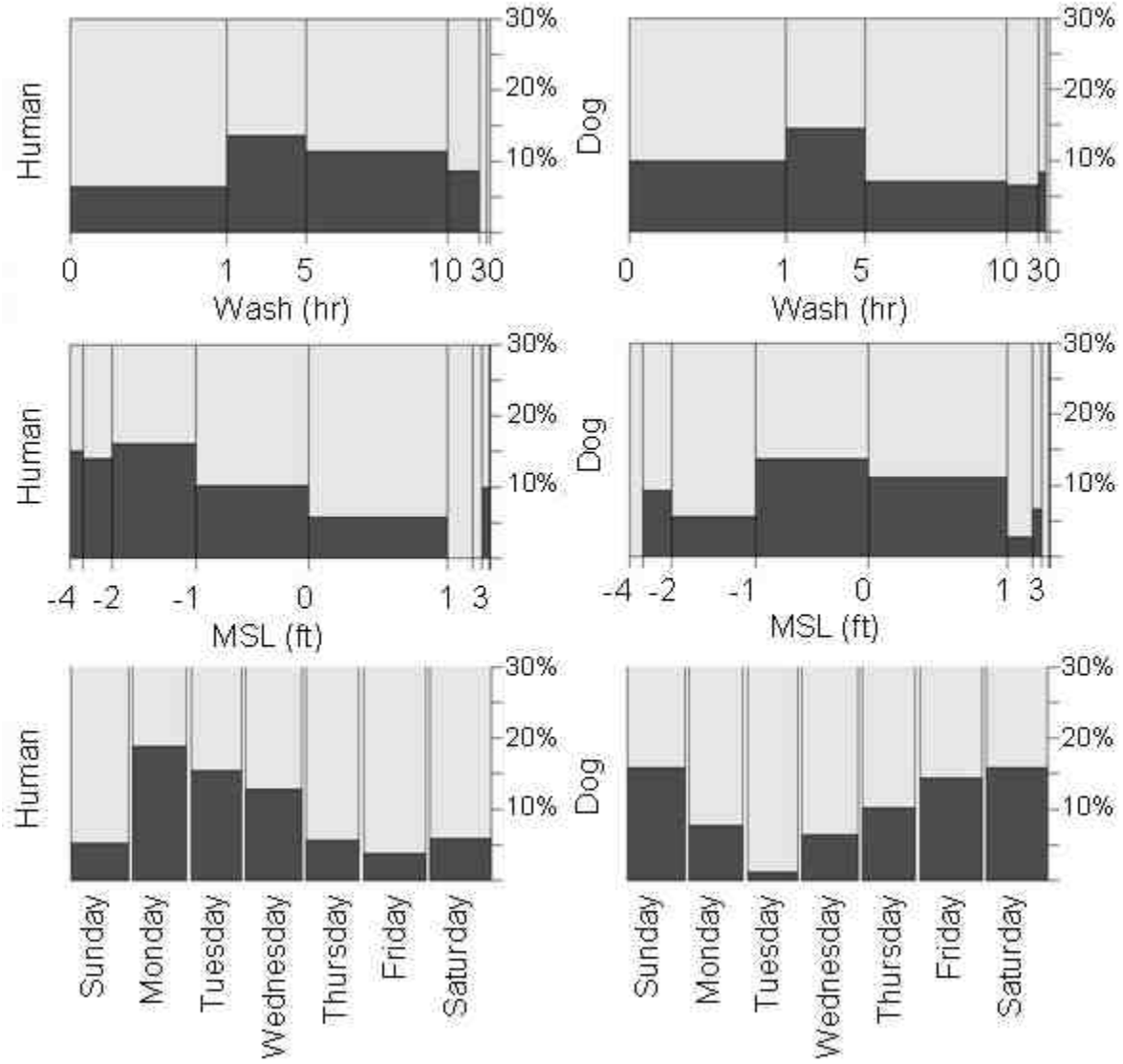


Figure 6.6.3-1. Visualizing and comparing significant human- and dog-specific *Bacteroides* PCR predictors (Wash, MSL and Day) for sampling sites PB3-PB5. The width of each bar indicates the number of samples in each category. The height of the black bar shows the proportion of samples with a positive result for human- or dog-specific *Bacteroides* as indicated by the scale on the right of each plot.

We also compared the *Bacteroides* PCR method for FST (section 6.4.3) with the ribotyping of *E. coli* strains (section 6.4.6). Because the sensitivity and sampling methodology for the two FST methods is very different, a comparison of percent contributions would not be informative. The *Bacteroides* method utilized a 500 mL sample and tested the entire sample for a specific marker. The ribotyping method collected 1 to 4 *E. coli* strains from each sample and matched them to a

library of *E. coli* from known sources. Consequently, we tested the hypothesis that the presence of an *E. coli* strain matching a dog source should predict a positive dog-specific *Bacteroides* PCR result from a sample collected at the same time and place. This was tested by examining the number of samplings positive for both dog-specific bacteria, *E. coli* and *Bacteroides*. Out of 133 sample times where an *E. coli* strain was collected matching a dog, canine or coyote source, only 15 also returned a positive dog-specific *Bacteroides* result. This is not a significant correlation (Chi squared p = 0.7). Given that the two samples (one for *Bacteroides* DNA and one for *E. coli*) were collected in different bottles as much as a minute apart, this result does not necessarily indicate a flaw in either FST method.

6.6.4. Statistical Models for predicting Pathogen levels

To determine if any pathogens were appearing in concert with FIB counts pairwise correlations between pathogens and FIB counts were computed for each site. Based on a sample size of 24, correlations with magnitude larger than 0.337 were significant at the 0.05 level using Pearson's z-test for correlation. Because of the large number of pairs, the Bonferroni adjusted level 0.05 tests are significant for correlations greater than 0.464 in magnitude.

After Bonferroni corrections, *Campylobacter* spp., *P. aeruginosa*, and *V. vulnificus* showed significant correlations to Ent counts, but only at the L1 site (Table 6.6.4-1). Pathogen levels at the PB4 site did not correlate to any FIB counts.

Table 6.6.4-1. Coefficients from pairwise comparison testing of pathogen correlations to FIB at each site. Significant results are in bold, based on a Bonferroni adjusted significance level.

	PB4			L1		
Organism	**TC**	***E. coli***	**Ent**	**TC**	***E. coli***	**Ent**
Aeromonas spp.	–0.214	–0.192	0.013	–0.111	–0.039	–0.030
Campylobacter spp.	0.174	0.192	0.031	0.351	0.342	**0.567**
Pseudomonas spp.	–0.132	–0.116	0.052	–0.254	–0.086	–0.191
P. aeruginosa	–0.400	–0.407	–0.222	0.245	0.387	**0.467**
Salmonella spp.	–0.005	–0.015	0.111	0.145	0.260	0.326
Shigella spp.	0.293	0.338	0.108	0.281	0.228	–0.171
V. parahaemolyticus	0.018	0.042	0.231	–0.065	0.135	–0.216
V. vulnificus	0.260	0.200	0.245	0.201	0.294	**0.547**

To examine relationships between pathogen counts and environmental variables each log transformed pathogen concentration was regressed by Site (PB4 vs. L1), Sal = Salinity (millisiemens/centimeter), Turb = Turbidity (Nephelometric Turbidity Units), UV = absorbance a 254 nanometers (absorbance), Temp = water temperature (degrees centigrade). No easily discernable pattern was visible from the few significant predictors of pathogen abundance (Table 6.6.4-2) with the possible exception of turbidity predicting levels of both types of *Pseudomonas*.

However the coefficient is so low as to make one wonder about the use of this variable as a predictor.

Table 6.6.4-2. Coefficients, t-values and p-values for the variables included in a model for predicting pathogen counts. The model accounts for differences by sampling site. Significant variables are denoted by boldface p-values. Water temperature showed no significant correlation to pathogen levels.

Pathogen	Variable	Coef	t	p
.Aeromonas spp.	Sal	-0.051	-2.061	**0.045**
	Turb	0.018	0.904	0.371
	UV254	1.598	0.892	0.377
Campylobacter spp.	Sal	0.020	0.954	0.345
	Turb	0.016	0.984	0.330
	UV254	1.918	1.313	0.195
Pseudomonas spp.	Sal	-0.010	-0.800	0.428
	Turb	0.023	2.322	**0.025**
	UV254	-2.230	-2.522	**0.015**
P.aeruginosa	Sal	-0.009	-0.625	0.535
	Turb	0.025	2.132	**0.038**
	UV254	0.194	0.181	0.857
Salmonella spp.	Sal	-0.004	-0.326	0.746
	Turb	0.014	1.428	0.160
	UV254	-0.504	-0.544	0.589
Shigella spp.	Sal	0.026	1.213	0.231
	Turb	-0.023	-1.424	0.161
	UV254	0.422	0.282	0.779
V. parahaemolyticus	Sal	-0.031	-1.287	0.204
	Turb	-0.014	-0.774	0.442
	UV254	-1.056	-0.619	0.539
V. vulnificus	Sal	0.091	3.615	**0.001**
	Turb	0.013	0.659	0.513
	UV254	2.915	1.635	0.109

7. Conclusions

7.1. Fecal Contamination on Pismo Beach

The main purpose of this study was to determine the source of fecal contamination resulting in frequent posting of Pismo Beach with bacteria level warnings by the SLO-CPHD. We investigated the levels of FIB, chemical and physical parameters, as well as the presence of other fecal source marker bacteria intensively during the summer of 2008 and across the year of May 2008 to May 2009. The data collected in this study clearly show that the main source of fecal contamination on the beach is bird droppings near the pier.

7.1.1. Pigeons Account for the High FIB Counts at Pismo Beach

The most direct evidence for birds in general and pigeons more specifically as the source of high FIB counts comes from the ribotyping FST method employed by IEH. Nearly 40% of the *E. coli* strains collected in this study matched the WildBird category of fecal sources (Table 6.4.6-1 and 6.4.6-2), and *E coli* strains with the same pigeon-specific ribotype were collected twelve times from within 150 meters of the pier (section 6.4.6). Indirect evidence for pigeons as a source of FIB counts also comes from several angles. First, the sampling sites nearest the pier consistently showed the highest counts of FIB, whether sampled weekly, daily or hourly (Tables 6.4.2-1, 6.4.2-2, 6.4.2-3, 6.4.2-4). AB411 exceedences were also most common in close proximity to the pier (Figures 6.4.2-1, 6.4.2-2, 6.4.2-3). In addition, volunteer observations of the highest count of bird droppings within 150 meters of the pier (Table 6.5-1). A one-time survey estimated the size of the Pismo Beach pigeon flock at well over 400 birds with more than 200 pigeon nests on the structural members of the pier itself. Additional correlations to oceanographic conditions also corroborate the pier as a source of FIB. Both wave direction and current direction influenced FIB counts around the pier in such a way as to make it clear that water movements push high concentrations of FIB away from the pier as the main source of fecal contamination (section 6.6.1., Figures 6.6.1-2 and 6.6.1-3). Finally, measuring the time since a tide last washed the part of the beach being sampled (the Wash variable) was an excellent predictor of FIB count, indicating that deposition of fecal matter on the beach itself was the predominant contamination mode (Table 6.6.1-1 and Figure 6.6.1-1). All these pieces of information taken together present a convincing argument for the pigeon flock at the Pismo Beach pier as the main source of fecal contamination in the surrounding ocean water.

7.1.2. Human, Dog, Cow, Horse and other Fecal Sources

We also tested for Human, Dog, Cow and Horse-specific *Bacteroides* markers using PCR (section 6.4.3). We saw no evidence of horse fecal contamination in any of the samples we tested although this may have been due to a low detection limit (Table 5.2.2-1) as horses were observed on the beach by the volunteers (Table 6.5-2). As expected, evidence of cow fecal contamination was common in the creek samples taken during rain events, was only rarely seen in beach samples, and almost never observed in samples taken near the pier (Table 6.4.3.1-2). The comparison of *E. coli* strains collected at Pismo Beach to a library from known fecal sources also confirmed the presence of human, dog, cow and horse fecal contributions to the mix at the beach (Table 6.4.6-1), though all except dog contributions were quite small.

While many samples were positive for Human and Dog *Bacteroides*, we found no evidence for dog or human influence on FIB counts (section 6.6.1). In addition, these assays were sensitive enough to detect less than a tenth of a gram of fecal matter in a liter of ocean water (Table 5.2.2-1), far less than what is required to detect FIB from the same source. However, it is clear that both human and dog feces are making it into the ocean at Pismo Beach. About 20% of the *E. coli* strains collected at the beach were matched to dog sources (Table 6.4.6-1). Volunteers mention witnessing at least 3 instances where owners did not pick up after dogs and kids in diapers were seen almost every other day in the summer (Table 6.5-2).

We saw some other indications as to how these fecal sources may be entering the ocean. Samples positive for dog feces were more common on the weekends (Figure 6.6.3-1), although volunteers did not see significant differences in the number of dogs on the beach from day to day (section 6.5). We also saw the same *E. coli* strain ribotype collected at the beach 76 times, which may indicate a local population of dogs repeatedly leaving contributions on the beach.

Intriguingly, samples positive for human feces were more common in the middle of the week though why this was true remains mysterious. It is also very clear that large crowds at the beach can result in an increase in the frequency of human fecal contamination. A five day window on each side of the July 4th holiday in 2008 netted a large set of human *Bacteroides* positive samples – even extending to samples taken from the ocean beyond the surfzone (Figure 6.4.3.1-1).

While many other fecal sources were implicated from the ribotyping study (Table 6.4.6-1), including some rather dubious sources for a beach (bear, rabbit, opossum), none were represented in high enough proportions to be considered in management plans for lowering FIB counts at the beach.

7.1.3. Rain Events

As expected, FIB counts in the creeks and lagoon were very high during and after rain events (Table 6.4.2-5). However, given the small amount of rain that fell, and given that the lagoon did not breach the dune and empty into the ocean until the last two rain events sampled, it is perhaps unsurprising that rain had little influence on the FIB levels across the beach sites and only had a significant influence on Ent counts when turbidity was also taken into account (Table 6.6.1-1). This could change if storms with more extensive amounts of precipitation were measured. Although all FIB counts were highest to the north of the pier during rain events (Table 6.4.2-5), this was not a significant difference when variation due to other parameters was taken into account.

7.1.4. Pismo/Grover/Oceano Joint Outflow

Based on the REMUS missions run around the outfall and along the beach at the Pismo Beach pier and the dilution estimates from the data collected on these missions (Table 6.2.1-1), there is no indication that, on the three days sampled, the influence of the outfall extended beyond 500 m from the source. In fact, much of the dilution of the effluent took place within 100 m of the source. Therefore, it is very unlikely that the outfall is a source of contamination on Pismo Beach around the pier, which is over 4 km away.

Another investigation of a nearby ocean outfall corroborates these results. In a recent report on the Montecito Outfall near Santa Barbara (Ohlmann et al, 2010) similar dilution results were reported with 100 to 900 fold dilutions of effluent occurring within 500 m of the outfall diffuser. The report concludes that the Montecito Outfall, located ~500 m offshore at an 11 m depth, does not have a measurable effect on the microbial populations on the shoreline. Measureable effects were occasionally seen directly over the outfall.

Similarly, this study also found a few positive results for both human and dog *Bacteroides* at site O1 on the surface over the outfall (Table 6.4.3.1-1). During the three REMUS missions, effluent mixed efficiently in the bottom waters and reached a dilution point at which effluent water no longer mixed vertically upward. This produced a layer of mixed effluent water at a depth of approximately 10-14 m. It should be emphasized that while the spatial extend of this layer was restricted in these three missions, there was variability in the vertical extent of mixing and an occasional surfacing of mixed effluent water is likely in the area directly above the outfall.

7.2. Methodology for Source Tracking in Beach Environments

As shown by the comparison with historical data taken at Pismo and Huntington beaches, the best way to study fecal contamination on an ocean beach is to sample in such a way as to cover many different tides. This in combination with good placement of sampling sites allowed us to pinpoint sources of FIB at Pismo Beach both geographically and temporally with respect to the tide cycle.

PCR for source marker bacteria also proved an effective method for tracking fecal contamination in ocean water samples. However, some care must be taken to check specificity and detection limits. For example, we discovered that the PCR primers purported to be for "Dog-specific" *Bacteroides*, in fact produced false positive results with 7 of the 10 cat feces we tested (Table 5.1.1-1). In addition, the response to species specific PCR varied with the source of feces. For example some humans do not harbor the "Human-specific" *Bacteroides*. This makes comparison of quantitative *Bacteroides* measurements to FIB levels very difficult. Lastly, knowledge of detection limits is important for understanding the data gathered by species specific *Bacteroides* PCR. We discovered that a large amount of horse fecal material was required to obtain a positive PCR result and that may have caused a lack of evidence for horse fecal contamination in this study, even though Pismo Creek services a watershed with several ranches and horses were observed on Pismo Beach.

Because no source-specific markers currently exist for bird fecal sources, the use of IEH's massive *E. coli* strain library for matching ribotypes has an advantage over PCR-based source marker methods. However, our study was presented with some problems when employing this method. First, our blind test with *E. coli* strains isolated from known sources resulted in very few matches with the IEH library and two of the three matches returned were incorrect (section 5.5). It's interesting to note that one of these mismatches (dog matched to cat) was also an issue with the *Bacteroides* PCR method. While an explanation for this result was offered by IEH, we cannot confirm it without more information. This means conclusions drawn from the IEH data should be viewed with caution. Fecal sources such as bear, opossum and rabbit, which were found infrequently and seem very unlikely in a beach water sample with no rain to wash feces into the ocean should probably be ignored. However, this method provided some key pieces of

information that tied birds in general and pigeons in particular to the deposition of *E. coli* strains into the ocean at Pismo Beach and the data as a whole should not be discounted.

TRFLP proved ineffective as a method for fecal source tracking. There are too many bacteria already present in seawater so fecal contamination must be quite extensive to detect via TRFLP (section 5.2.3). Similarly, the detection of Enterovirus was deemed ineffective as a method for tracking human fecal contamination since a massive sewage spill would be required to detect the virus in ocean waters (section 5.2.1).

7.3. Pathogens in the Water at Pismo Beach

The pathogens we tested for were quite common in both the obviously murky waters of the Pismo Creek lagoon as well as the clear waters next to the pier on the beach. In many cases, for healthy individuals, the amount of pathogens in the samples would require ingestion of large volumes of seawater to risk infection. However, some pathogens, such as *Pseudomonas*, *Giardia* and *Cryptosporidium*, did present a risk at the levels we detected. Swimming in the lagoon would obviously expose the swimmer to a reasonably high risk of disease. However, pathogen levels at PB4 were rarely high and generally significantly lower than in the lagoon (Table 6.4.4-1) so the risk of disease from swimming next to the pier would be orders of magnitude lower.

Given that pigeon feces harbor some of the pathogens we tested for (Table 6.4.4.7-1) it does make sense to post warnings on the beach when FIB counts are high due to pigeon droppings. However, not all of the pathogens we tested for were correlated with high FIB counts. In fact, the two most common pathogens found in pigeon feces, *Aeromonas* spp. and *Pseudomonas* spp., were not correlated to FIB counts at all. Perhaps these bacteria die off in seawater at a different rate than do FIB. The fact that the levels of these two pathogens were correlated with each other at PB4 (Table 6.4.4-3) lends support to the idea that pigeons are the main source of contamination at that site. Interestingly, levels of *Campylobacter* spp., a pathogen known to be carried by birds, correlated well with FIB counts. However, very low levels of *Campylobacter* spp. were found at PB4 and pigeons do not appear to be common carriers (Table 6.4.4.7-1).

7.4. Prospects for a Rapid Human Source Detection Kit

The team at Advanced Liquid Logic made good progress toward building a kit for the rapid detection of Human *Bacteroides* in seawater, but we estimate another year of work is required before such a kit can be brought to market.

8. Recommendations

8.1. A Plan for Pismo Beach

If it were possible to remove the entire flock of pigeons from around the Pismo Beach pier, it would probably reduce the number of AB411 exceedences at the PB4 site to levels closer to those seen at PB3 or PB5. Several alternatives exist to at least begin to reduce the number of

pigeons using the pier as a roosting place. Some possibilities include: capture and remove or destroy the existing flock; net off or somehow make the underside of the pier inaccessible to roosting birds; feed birth control laced pigeon food to the flock to allow them to naturally dwindle in numbers over time. A combination of some of these methods may prove the most effective. In addition, a well designed pre- and post-treatment study would help to demonstrate the effectiveness of any approach taken.

In addition, since we have shown that both human and dog feces are getting into the beach water, it may be important to consider ways to keep this to a minimum. Possibilities include increased restroom access for swimmers, especially during high beach visitor times and an increased presence on the beach to enforce dog dropping pickup laws more strictly or with higher fines for failure to comply.

Lastly, it may be prudent to post the dangers of swimming in the Pismo Creek lagoon to ensure the public is informed about the risks to one's health inherent to those stagnant and pathogen-filled waters.

8.2. Beach Monitoring and Source Tracking Recommendations

The most important conclusion to come out of this study is the understanding that FIB counts are highly correlated to the tide cycle and the time a section of beach has been exposed since the tide last covered it. This study and the study at Huntington Beach (Rosenfeld et al 2006), both point to deposition of fecal material on the beach sand as the main source of FIB in the surfzone at California beaches. Recent studies have also shown the importance of FIB in beach sand (Yamahara et al., 2007). Consequently, the way in which sampling times fit into the tidal cycle has clear implications for the public health monitoring of beaches and for future studies on the dynamics and sources of bacterial deposition in beach waters. For example, choosing to sample based on an incoming tide, past the half way point to full, would ensure consistently higher FIB counts, and provide a better estimate of the worst contamination conditions a beach may present. At a minimum, similar tide cycles should be sampled when monitoring beaches for bacterial levels so that one is collecting comparable data when making choices about posting a beach for excessive bacterial contamination. If a specific time of day is required for the logistics of sampling, the day a beach is sampled could be varied throughout the year to ensure a more consistent tide level is sampled.

For tracking the sources of FIB, several recent reports have suggested a multi-level approach using standard FIB counts coupled with FST methods (Boehm et al., 2003, Noble et al, 2006). Three FST methods were investigated in this study and while the use of TRFLP proved ineffective, it's possible to provide some recommendations for the other two methods used. Source-specific PCR provided very useful information in this study. With careful consideration for the cautions about detection limits and specificity mentioned in section 7.2, excellent data on the presence of some fecal sources was relatively easy to obtain. However, PCR is still a relatively expensive, expert driven method that would not be in easy reach of most beach communities without State or Federal funding assistance. We hope that new technologies soon mature, similar to the device developed by ALL, which will produce a non-expert kit for detecting important fecal source markers. Massive *E. coli* strain library matching also provided some key data in this study. Again, there are cautions associated with using this method and

quality control tests need to be formulated to determine how well a library will work in any particular study.

In summary, the choice of FST method used in a beach water quality study should be judged on an estimate of the fecal sources that could be involved. However, until a good PCR-based marker is devised for bird feces a combination of source markers and *E. coli* strain library matching will probably be the most informative.

8.3 Future Research Directions

This study has highlighted several deficiencies in current technology that should be addressed in the near future. First, there is a clear need for a rapid test (less than 2 hrs) for FIB in recreational waters. We understand that the US-EPA is making strides toward certifying a PCR based method and hope it is approved soon since it will allow meaningful postings of recreational waters. This is also an excellent target for the development of non-expert kits that would allow beach communities to inexpensively monitor their own waters.

Source tracking technology also needs some continued research. For example, it is important to know how long a sample of Human *Bacteroides* will remain detectable when exposed to seawater. PCR will detect live and dead cells so the use of *Bacteroides* PCR could be misleading if the signal outlasts FIB counts or pathogens in the same environment. Similarly, it would be useful to understand the relationship between FIB counts and species-specific *Bacteroides* levels in populations of host animals so this method can be related to the bacterial counts more commonly used by regulatory agencies.

Work should also continue on *E. coli* strain library matching technology. More information should be gathered on the distribution of *E. coli* strains in birds and mammals, the proportion of transient strains that show up in multiple species, and the optimal number of strains a library must possess to be effective. It would also be useful for California to have a regionally specific strain library available to help beach communities.

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